

From Cortical Excitation To Cognition: The Case Of Mathematics



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List of abbreviations

Abbreviation	Short for	Context
A-tDCS	Anodal tDCS	Noninvasive electrical brain stimulation
BET	Brain extraction tool	Brain imaging analysis
C-tDCS	Cathodal tDCS	Noninvasive electrical brain stimulation
Cr	Creatine	Brain metabolite
CSF	Cerebrospinal fluid	Brain tissue
DBS	Deep brain stimulation	Technique
DLPFC	Dorsolateral prefrontal cortex	Brain anatomy
fMRI	Functional magnetic resonance imaging	Brain magnetic resonance imaging
FSL	Oxford Centre for Functional Magnetic Resonance Imaging of the Brain's Software Library	Brain imaging analysis
GABA	Gamma-aminobutyric acid	Brain metabolite
GM	Gray matter	Brain tissue
Hz	Herz (unit)	Electrical current
IFG	Inferior frontal gyrus	Brain anatomy
IHI	Interhemispheric inhibition	
IOG	Inferior occipital cortex	Brain anatomy
IPS	Intraparietal cortex	Brain anatomy
M1	Primary motor cortex	Brain anatomy
MEGA-PRESS	Mescher-Garwood Point RESolved Spectroscopy	Magnetic resonance imaging
ml	Mili liter	Volume unit
mmol/l	Mili moles per liter	Unit
MRI	Magnetic resonance imaging	Magnetic resonance imaging
MRS (1H-MRS)	Magnetic resonance spectroscopy	Magnetic resonance imaging
MVS	Multi-voxel spectroscopy	Magnetic resonance imaging
NAA	N-acetyl aspartate	Brain metabolite
NIBS	Noninvasive brain stimulation	Noninvasive brain stimulation
NIRS	Near-infrared spectroscopy	Brain imaging
OVS	Outer-volume suppression	Magnetic resonance imaging
PPC	Posterior parietal cortex	Brain anatomy
PRESS	Point-Resolved Spectroscopy	MR spectroscopy sequence
rTMS	Repetitive transcranial magnetic stimulation	Noninvasive brain stimulation
SNR	Signal-to-noise ratio	Magnetic resonance imaging

SPECIAL	Spin-echo full-intensity acquired localized	Magnetic resonance imaging
SPSS®		Statistical analysis software
SVS	Single-voxel spectroscopy	Magnetic resonance spectroscopy technique
tACS	Transcranial alternating current stimulation	Noninvasive electrical brain stimulation
tDCS	Transcranial direct current stimulation	Noninvasive electrical brain stimulation
TE	Echo time	Magnetic resonance imaging
tES	Transcranial electrical stimulation	Noninvasive electrical brain stimulation
TMS	Transcranial magnetic stimulation	Noninvasive brain stimulation
TR	Repetition time	Magnetic resonance imaging
tRNS	Transcranial random noise stimulation	Noninvasive electrical brain stimulation
VAPOR	Variable pulse power and optimized relaxation delays	Magnetic resonance imaging
WM	White matter	Brain tissue

Summary

Excitatory and inhibitory neurons have important roles in learning and skill acquisition in the brain. Glutamate and gamma-aminobutyric acid (GABA) are the brain's major excitatory and inhibitory neurotransmitters in the brain, respectively. Until recently, the link between such neurochemicals and higher-order cognition could not be directly observed in the living human brain. With the advent of magnetic resonance spectroscopy (MRS), an MRI-based method to measure regional concentrations of a whole range of neurochemicals, it has become possible to link behavioral measures with glutamate and GABA. Here I investigated whether glutamate and GABA in a fronto-parietal mathematical brain network were associated with mathematical abilities in children, adults, and expert calculators, including an individual with prodigious calculation abilities. I found that the relationship differs as a function of the brain area (e.g. the hemisphere), age, gender, and ability.

Furthermore, regional levels of glutamate and GABA can be artificially modulated by transcranial electrical stimulation (tES), a non-invasive method to affect cortical excitability and increase the potential for plastic changes in the brain. As a possible neuro-enhancement tool, I investigated whether tES to frontal and parietal cortices during mathematical task execution can reliably improve complex arithmetic training effects, and whether such effects were accompanied by changes in regional concentrations in glutamate and GABA. In two double-blind, sham-controlled studies involving a five-day training paradigm, I could not replicate the exact results from a previous study. The previous study found beneficial effects of stimulation to bilateral dorsolateral prefrontal (DLPFCs), but not posterior parietal cortices (PPCs) on learning and transfer to novel task material using the same training paradigm. In a counter-balanced, sham-controlled, within-subjects design, I found that certain sub-tests improved when stimulating DLPFCs, while others improved when stimulating PPCs. In a subsequent between-subjects study, I found impairments in the group that received stimulation to PPCs. Moreover, I could not find changes in glutamate and GABA in the groups that received real, compared to sham stimulation. In a different paradigm, I also investigated whether an individual with exceptional calculation abilities, the 'lightning

calculator' G.M., could benefit from tES. In a sham-controlled within-subject single-case study across six testing sessions on two separate days, I found no improvement of G.M.'s calculation abilities. In order to test whether other expert calculators would show the same lack of an effect, I tested six postgraduate students in mathematics fields in an adapted but similar tES paradigm. These experts showed impairment in calculation performance under tES compared to sham.

The failure to replicate previous results and the impairments observed in two different samples suggest that the effects of tES on cognition are currently relatively unpredictable. Therefore, positive outcomes of individual tES studies should be interpreted with caution. MRS can be a useful tool to investigate brain-behavior relationships at a neuro-biological level and ideally, further research will demonstrate whether glutamate and GABA can be used as neural markers for poor cognitive abilities. Ideally, MRS will aid the diagnosis of cognitive difficulties at the neurochemical level, such that neuro-intervention can be targeted to enhance cognitive plasticity accordingly.

1 Introduction

Learning is a fascinating concept in cognition. Learning according to the Oxford dictionary is defined as “the acquisition of knowledge or skills through study, experience, or being taught” (OxfordDictionariesOnline, 2012). Learning can change the functioning and structure of the brain (Delazer et al., 2005; Driemeyer, Boyke, Gaser, Buchel, & May, 2008; Sampaio-Baptista et al., 2015; Sampaio-Baptista et al., 2014). For example, neuronal connections can change, or new neurons can form in the brain (neurogenesis), neuronal parts and other cells can increase or decrease (e.g. number and density of receptors, myelin, astrocytes and glial cells), and lastly, blood vessels providing nutrition to the cells can change in size and quantity (Zatorre, Fields, & Johansen-Berg, 2012). Neurotransmitters are messenger chemicals in the brain that are released by pre-synaptic neurons and affect post-synaptic neurons. The transmission process is a crucial part of neuronal communication. Neurotransmitters are crucial for brain changes: the more synchronized the firing of the cells, the stronger their interconnections become. The oldest idea of such “synaptic learning” was introduced by Donald Hebb in 1949 (Hebb, 1949). His statement “cells that fire together wire together” had a major influence and still remains prominent in the field of plasticity and learning. The terms ‘learning disability’ and ‘learning difficulty’ emphasize that learning processes can be impaired in certain individuals. Cognitive learning difficulties can lead the affected individual to be greatly compromised in fulfilling short-term and long-term tasks and goals of everyday functioning, which are otherwise easy for normal learners (Stein, Blum, & Barbaresi, 2011). Such learning difficulties have also been associated with atypical brain functioning and structural development (Kaufmann, Wood, Rubinsten, & Henik, 2011; Krain & Castellanos, 2006; Kucian et al., 2006; Park, Badzakova-Trajkov, & Waldie, 2012; Price, Holloway, Rasanen, Vesterinen, & Ansari, 2007; Proal et al., 2011; Rotzer et al., 2008; Rotzer et al., 2009; Rykhlevskaia, Uddin, Kondos, & Menon, 2009; Shaw et al., 2012; Silani et al., 2005). It has been suggested that learning difficulties may benefit from non-invasive brain stimulation, which may improve functioning of sub-optimal learning-related brain substrates and allow

for greater plasticity (Krause & Cohen Kadosh, 2013; Krause, Marquez-Ruiz, & Cohen Kadosh, 2013; Vicario & Nitsche, 2013a, 2013b). Before such effects can be investigated in groups of individuals with learning difficulties, it is important to investigate how non-invasive brain stimulation can improve cognitive learning in normal learners. The current thesis therefore focuses on the link between non-invasive measures of neurochemicals and learning in adults and numerical and arithmetic abilities. Furthermore, I investigated the effects of transcranial electrical stimulation (tES) on arithmetic learning and subsequently on whether changes in cognition are linked to changes in learning-related neurochemicals. A long-term goal of my approach is to investigate whether tES is a useful intervention for individuals with difficulties in mathematical learning, and whether we can identify and target the problem at the neurochemical level.

1.1 Cognitive enhancement

Due to the debilitating personal, medical and economic consequences of cognitive impairments in developmental disorders, aging, and neuropsychiatric diseases, the “boosting of the brain” has become widely popular in the scientific community and the general population. Innumerable companies have picked up on the need for improvement in cognitive abilities, including memory, attention, and addiction and are now marketing a variety of products. Besides pharmacological ‘solutions’, common strategies for neuro-enhancement are computer training or games, physical exercise, meditative programs, diets, mnemonic strategies, and brain stimulation (for a comprehensive review, see Dresler et al., 2013).

Transcranial electrical stimulation (tES) is a promising tool for neuro-intervention and is currently extensively researched to treat a variety of neurological, neuropsychiatric and neurodevelopmental disorders, such as stroke, neurodegenerative diseases, chronic pain, addiction, schizophrenia, attention-deficit hyperactivity disorder (ADHD), speech, reading, mathematics and perceptual disabilities, to name just a few (see Table 1.1) for some cognitive studies and e.g. Benninger et al., 2010; Boggio et al., 2012; Brunelin et al., 2012; Cohen Kadosh, Soskic, Iuculano, Kanai, & Walsh, 2010; Ditye,

Jacobson, Walsh, & Lavidor, 2012; Ferrucci et al., 2008; Fiori et al., 2011; Foerster et al., 2015; Fregni et al., 2008; Fridriksson, Richardson, Baker, & Rorden, 2011; Holland & Crinion, 2012. There are certain ethical questions that need to be considered for the practical use of tES in cognition. For example, besides the potential use of tES in individuals with learning difficulties, the method is also likely to be an attractive option for personal, commercial and governmental purposes. It is important to discuss the necessity for regulations before such methods are introduced into clinical settings (Cohen Kadosh, Levy, O'Shea, Shea, & Savulescu, 2012; Maslen, Savulescu, Douglas, Levy, & Cohen Kadosh, 2013). However, it is also important to understand the impact of cognitive developmental problems and the benefit of tES to improve the lives of individuals with such problems. The consequences of learning difficulties involve life-long struggles, such as increased rates of unemployment, reduced income and generally lower socioeconomic status (Parsons & Bynner, 2005; Stein, et al., 2011). For society, this entails an acceptance of the consequences of the resulting unemployment and coverage of loss of tax revenue, higher rates of drug abuse, crime, special education costs and treatment for mental consequences, including depression (Gross, Hudson, & Price, 2009). This is a significant financial burden to society and a long-term struggle for the affected individuals themselves. For instance, psychiatric diagnoses are fairly common in individuals with learning disabilities, which is likely associated with the lack of success, including frustration and stigmatization (Raskind, Goldberg, Higgins, & Herman, 1999). Therefore, it is important to address the developmental problem associated with atypical brain development, which is otherwise hard to tackle.

1.2 Mathematical abilities

Mathematical abilities involve a complex combination of dealing with knowledge, logic, numbers and quantities, and operators. For instance, in what we call number-space mapping, a horizontal line with a certain range (e.g. from 0-100, 0-1,000 or -1,000-1,000) is presented and the participant is required to indicate the relative position of a given number per trial on this line

(Siegler & Opfer, 2003). The smaller the difference between the correct and the given position, the higher the accuracy on this task is. In children, performance on this task predicts standardized mathematical abilities (Sasanguie, Gobel, Moll, Smets, & Reynvoet, 2013; Siegler & Booth, 2004; Siegler & Opfer, 2003). Training such abilities can even improve more general mathematical abilities in those with poor development of mathematical skills (Kucian et al., 2011).

Individuals with poor mathematical cognitive development often have a poor prognosis for employment and socio-economic status, unless they undergo successful intervention to enhance their performance in the deficient modality (Gabrieli, 2009; Rimrodt & Lipkin, 2011; Stein, et al., 2011). Therefore, it is hoped that tES can be a successful tool for neuro-intervention for developmental cognitive difficulties (Krause & Cohen Kadosh, 2013; Vicario & Nitsche, 2013a, 2013b). The core mathematics brain regions that are associated with poor mathematics development consist of a wide fronto-parieto-occipital network, with structural abnormalities, such as reduced grey matter in bilateral prefrontal and parietal areas, white matter reductions in right temporo-parietal networks, but also decreased functional activation in bilateral intraparietal sulci (IPS) while performing arithmetic tasks, which can already be observed in children (Kucian, et al., 2006; Mussolin et al., 2010; Price, et al., 2007; Rykhlevskaia, et al., 2009). Of main interest for the current work were dorsolateral prefrontal cortices (DPLFCs) and the posterior parietal cortices (PPCs), including the IPS (Figure 1.1). Due to the proximity to the scalp surface, these areas provided optimal possibilities for electrode placement and measurement of neurochemicals.

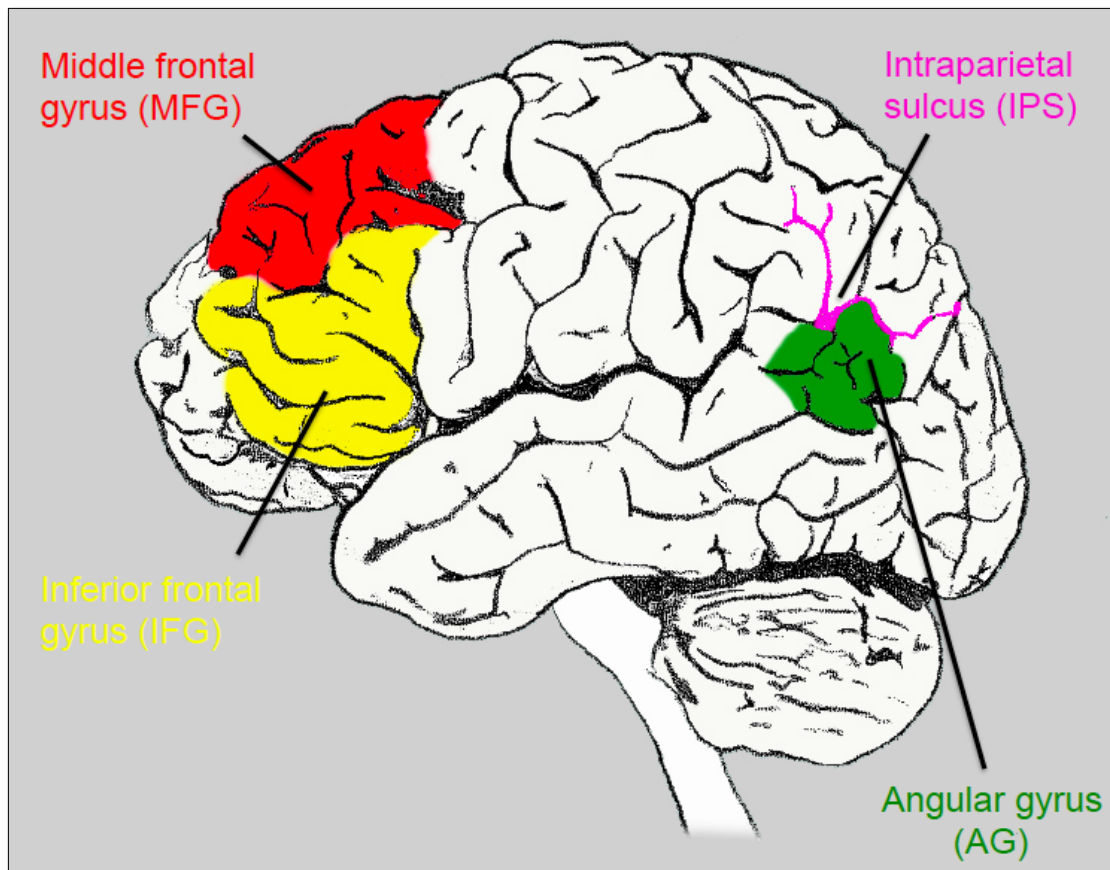


Figure 1.1

Brain areas important in number and arithmetic abilities (digitalized hand drawing). The middle frontal gyrus (MFG) contains the dorsolateral prefrontal cortex (DLPFC). The intraparietal sulcus (IPS) lies adjacent to the angular gyrus (AG), one of the major posterior parietal cortex (PPC) areas involved in mathematics (individual differences in brain anatomy can occur).

1.3 Transcranial electrical stimulation (tES)

The use of electrical current in order to treat various physical and mental medical conditions is not new. More than 2,000 years ago, humankind was not yet aware of the concept of electricity. However, they discovered the benefit of the electric current emitted by electric fish for medical purposes. Islamic and Greco-Roman writings report the treatment of pain, seizures and headaches. Holding the fish, such as the electric ray, catfish or eel, to the focus of pain or disease, the electricity was considered a cure to many such ailments (Finger & Piccolino, 2011). In the 18th century, the idea was rediscovered and mental illness including epilepsy and hysteria (intended for

women in particular) were targeted with electric currents (Gilman, 2008). Eventually, research on tES started a big boom in the past 20 years. After the now common use of implanted brain electrodes in what is called deep brain stimulation (DBS), neuropsychiatric disorders such as Parkinson's disease (PD), originating from structures deep in the brain, can now be successfully treated (Huys et al., 2012). The advantage of using surface electrodes that do not require the opening of the skull and risking damage on the brain is highly desirable and the hope is that we can treat many neurological disorders noninvasively with tES. TES can indeed induce long-term synaptic plasticity and enhance cognitive performance

(Cohen Kadosh, Dowker, Heine, Kaufmann, & Kucian, 2013; Miniussi et al., 2008; Nitsche et al., 2008). In tES, two or more electrodes are strapped to the head, and current flows from the anode to the cathode (Im, Park, Shim, Chang, & Kim, 2012; Nitsche & Paulus, 2001). The current is commonly delivered by a 9V battery pack. The current strength is very low, typically between 1 and 2mA. In order to increase the connectivity between the electrodes and the scalp surface, the electrodes are covered by saline-soaked sponges or gel, which reduces the risk of skin burns (Zaghi, Acar, Hultgren, Boggio, & Fregni, 2010). In most studies the positioning of the electrodes to stimulate the desired target brain areas is based on the international 10-20 system for EEG (electroencephalography) recording (see Figure 1.3) (Auvichayapat & Auvichayapat, 2011). The brain region is thereby more or less approximated, as we do not know the exact brain anatomy of the individual. However, due to the relatively large size of the electrodes (typically 16-35cm², with the exception of a more recent form of high-definition (HD-) tES and smaller electrodes 3.14cm²), such approximation is sufficient to direct the current to the area of interest. The typical stimulation duration in cognitive research is between 10 and 30 minutes, which is currently considered safe at intensities of up to 2mA (Fregni et al., 2014). It is important to note that both duration and intensity can reverse the beneficial effects to certain brain functions, the mechanism of which is currently not fully understood. I have previously hypothesized that the mechanism of action depends on the change in cortical excitation and inhibition (E/I) (Krause, et al., 2013). Alternative and

complementary theories exist and are discussed in (Bestmann, de Berker, & Bonaiuto, 2015).

TES is also a very attractive research tool, as it has a reliable and almost indistinguishable sham (placebo) condition (Gandiga, Hummel, & Cohen, 2006). This feature is currently not provided in other techniques to the same degree, such as transcranial magnetic stimulation (TMS). In TMS, magnetic pulses administered to the scalp surface cause a tapping sensation and loud clicks, and can even cause muscle contractions by inducing action potentials in the muscles under the stimulation site (Abler et al., 2005). Such contractions can be highly distracting and discomforting, and can thereby affect the participant's performance, in particular on cognitive tasks. It also makes the real stimulation easily distinguishable from the sham condition. TMS is not directly relevant for the current work, but for more information, see (Wagner, Valero-Cabre, & Pascual-Leone, 2007). The tES sham mode corresponds to the same parameters as the real stimulation settings, but is turned off after 30 seconds. It thereby mimics the skin sensations of the stimulation (e.g. tingling; see Table 1.2).

Since even under real tES the skin sensations occur only initially, and disappear subsequently, the individual receiving the stimulation is usually unable to tell what treatment condition they are in (Ambrus, Paulus, & Antal, 2010; Fertonani, Ferrari, & Miniussi, In Press). Due to the possibility to use preset stimulation parameters, experimenter blinding is also possible, which is highly desirable for double-blind sham-controlled studies. All tES studies in the current thesis involved such double-blind sham-controlled designs.

One of the most critical features of tES to consider for research is that the mere administration of the current will not induce any behavioral changes. While TMS induces action potentials in the neurons of the stimulated brain area with the corresponding direct behavioral result, tES needs to be paired with a task or training that engages the stimulated brain area (Reis & Fritsch, 2011). TES can enhance cortical excitability and thereby passively facilitates task performance. Such enhanced excitability during cognitive engagement allows for greater plastic changes, which may improve learning and performance in general. TES therefore affects the potential for plastic changes, but does not induce plastic changes by itself if the participant is at

rest while being stimulated (Cappelletti et al., 2013; Cohen Kadosh, et al., 2012). In tES, there are different forms of current. While I focused on one form of tES (namely transcranial random noise stimulation; tRNS), below I will briefly review the major tES methods used in the field.

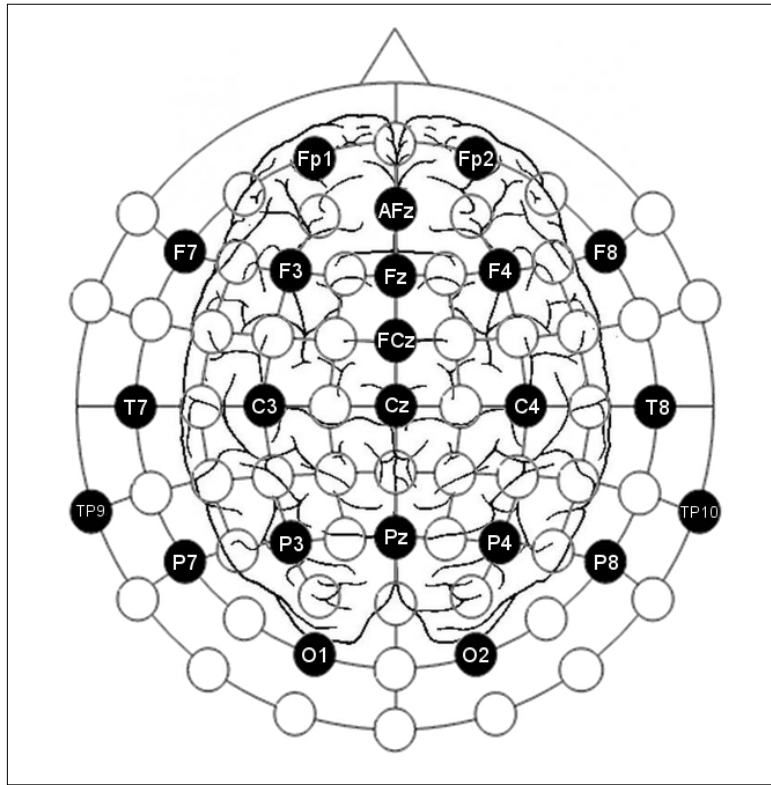


Figure 1.2

The international 10-20 system for EEG recording overlaid on the approximate brain areas to be stimulated using tES (nose on top). Empty circles represent the extended 10-20, also called 20-20 system, a denser way of mapping electrodes. The latter, however, is not relevant in the current thesis. Figure from (Krause, Looi, & Cohen Kadosh, 2014).

1.4 Forms of tES

1.4.1 Transcranial direct current stimulation (tDCS)

The most common and well-known form of tES is transcranial direct current stimulation (tDCS). Here, the current flows from the anode to the cathode, inducing mainly excitatory effects under the anode (anodal tDCS, A-tDCS), and inhibitory effects under the cathode (cathodal tDCS, C-tDCS) (Nitsche, Nitsche, et al., 2003; Nitsche & Paulus, 2000, 2001). At the cellular level, A-

tDCS depolarizes and C-tDCS hyperpolarizes the neurons in the stimulated area under the electrodes. Due to these opposing effects, tDCS is said to be 'polarity-specific'. The placement of the electrodes needs to be carefully considered and there are several different options (see Table 1.1). For instance, one can stimulate one area and simultaneously inhibit another, such as the same region in the contralateral hemisphere. Depending on the role of each brain area in the training task administered, reversing the hemispheric pattern can also reverse the cognitive effect. For example, cognitive performance can improve when stimulating right and inhibiting left parietal cortices, while the opposite configuration can even reduce performance on a cognitive training task (Cohen Kadosh, Soskic, et al., 2010). Another possibility is to stimulate one brain area using the anode and to place the cathode over a neutral area as a reference electrode. This is often either the contralateral supraorbital area (above the eyebrow on the forehead) or the vertex (the midpoint on top of the head) (DaSilva, Volz, Bikson, & Fregni, 2011). Finally, certain studies place the reference electrode on the cheek (buccinators muscle), the chin or the shoulder (deltoid muscle) (Im, et al., 2012). However, for the extracephalic electrode placement it is important to note that the current strength induced in the target brain area is reduced with an increasing inter-electrode distance, and therefore the current intensity needs to be adjusted accordingly (Moliadze, Antal, & Paulus, 2010). For these reasons, the most commonly observed placement involves either bilateral or supraorbital referencing strategies.

1.4.2 Transcranial random noise stimulation (tRNS)

In transcranial random noise stimulation (tRNS) a spectrum of random frequencies between predefined boundaries is induced by both electrodes simultaneously. Generally inducing cortical excitability, high-frequency tRNS (Hf-tRNS) lies between 101 and 640Hz, low-frequency (Lf-tRNS) between 0.1-100Hz, and full spectrum between 0.1-600Hz (Terney, Chaieb, Moliadze, Antal, & Paulus, 2008). A single session of 10min tRNS can increase neuronal excitability for up to at least 60 minutes after cessation (Terney, et al., 2008). This has important implications for research designs and clinical

use, as the brain continues to be plastic for longer than the stimulation duration, such that the duration itself can be relatively short.

Since both electrodes induce unspecific noise, tRNS is not polarity-specific. This also means that we can stimulate two (or more) brain areas at the same time with the same effects at each electrode. The putative mechanism underlying the excitability increases is called 'stochastic resonance'. According to this theory, the neuronal sensitivity to stimuli below the firing threshold becomes enhanced by the induced noise (Fertonani, Pirulli, & Miniussi, 2011). If the right amount of noise is induced, neurons with low levels of input, which would otherwise remain silent, can now fire during task execution. A common intensity for tRNS is between 1 and 1.5mA. Higher intensities can lead to a reversal of the beneficial effect and impair the neuronal response to the stimulus amongst the overbearing input of the noise (Moss, Ward, & Sannita, 2004). The electrode placement can be treated similarly to the management in tDCS, however, stimulating bilateral brain areas at the same time is interesting for certain research questions. Generally, tRNS has a higher perceptual threshold for skin sensations, such that it provides even more effective subject-blinding than tDCS (Ambrus, et al., 2010). Most importantly, tRNS has been shown to have superior effects compared to tDCS in certain cases (Fertonani, et al., 2011).

1.4.3 Transcranial alternating current stimulation (tACS)

The difference between the alternating current (tACS) and tRNS is that tACS involves a sine wave-shaped current at a fixed frequency. The effects are not as well-understood and straightforward as in the tDCS and tRNS, but generally, different frequencies have different effects on neuronal firing patterns, which can be observed in cortical oscillations and synchronicity (e.g. (Antal & Paulus, 2013; Kanai, Chaieb, Antal, Walsh, & Paulus, 2008; Santarnecchi et al., 2013; Thut, Miniussi, & Gross, 2012). TACS has been used more and more for cognitive abilities, but finding the optimal frequency is often a hit or miss (Herrmann, Rach, Neuling, & Strüber, 2013; Kanai, et al., 2008; Santarnecchi, et al., 2013). One of the disadvantages of tACS is its side effect to induce phosphenes, the false perception of light flashes at the cortical level, which is not a real stimulus (Antal & Paulus, 2013; Kanai, et al.,

2008; Turi et al., 2013). Both the phosphenes and skin perceptions induced by tACS will affect adequate subject-blinding, depending on the frequency (Turi, et al., 2013). However, with careful consideration of the stimulation parameters, these can generally be avoided (see Davis, Gold, Pascual-Leone, & Bracewell, 2013).

1.4.4 Other forms of tES

More recent advancements have introduced high-definition (HD-) tDCS, which involves much smaller surface areas of the disk-like electrodes (e.g. 4mm radius), aiming for more focal effects (Datta, Elwassif, Battaglia, & Bikson, 2008). Such methods may be valuable for certain patient groups or highly specific hypotheses. However, it requires the use of complex and expensive neuronavigation, in which an acquired structural MRI scan is used to find the correct scalp position for the electrode placement. It is therefore only necessary or suitable for certain kinds of research questions.

Transcranial pulsed current stimulation (tPCS), previously called cranial electrical stimulation (CES), is similar to tACS in that it uses wave-like current with typically 100Hz (reported between 0.5Hz and 167kHz) between 50 μ A – 5mA. The frequency can be fixed or random between predefined boundaries and can modulate cortical oscillations (Castillo Saavedra et al., 2014). However, the placement of the rather small electrodes (0.1cm² – 35cm²) inside or around the ears (e.g. the ear lobes or mastoid processes) allows the current to reach deep brain structures (Datta, Dmochowski, Guleyupoglu, Bikson, & Fregni, 2013; Zaghi, et al., 2010). Due to the indirect nature of the current approximating deeper structures, it has been suggested to be more adequately named a type of peripheral nerve stimulation (Zaghi, et al., 2010). Even though this method is not directly relevant to the current thesis work, one study on mathematics cognition has used tPCS (Morales-Quezada et al., 2014).

Electrode position	Function	Brain area targeted	Stimulation	Reference
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Language				
F3	Vocabulary and syntax	Left DLPFC	A-tDCS (reference contralateral supraorbital)	(Schneider & Hopp, 2011)
FC5	Speech, naming	Left inferior frontal gyrus (IFG)	A-tDCS (reference contralateral supraorbital)	(Holland et al., 2011)
CP5	Word retrieval	Superior temporal gyrus (STG; Wernicke's area)	A-tDCS (reference contralateral supraorbital)	(Fiori, et al., 2011)
CP5	Associative language learning	Left posterior perisylvian area (Wernicke's)	A-tDCS (reference contralateral supraorbital)	(Floel, Rosser, Michka, Knecht, & Breitenstein, 2008)
CP5-CP6	Speech	Left superior temporal gyrus (STG; Wernicke's area)	Left-anodal, right-cathodal (LARC)	(You, Kim, Chun, Jung, & Park, 2011)
T7/TP7-T8/TP8 (extended 10-20 system)	Word reading efficiency	Left posterior temporal cortex	Left-anodal, right-cathodal (LARC)	(Turkeltaub et al., 2012)
Memory				
P3-T5; P6-T4	Word	Bilateral	A-tDCS	(Ferrucci, et

	recognition memory and visual attention	temporoparietal junctions		al., 2008)
T3-T4	Visual recognition memory	Bilateral temporal lobes	A-tDCS (external reference right deltoid muscle)	(Boggio, et al., 2012)
Working memory				
F3	3-back task	Left DLPFC	A-tDCS (reference contralateral supraorbital)	(Ohn et al., 2008; Teo, Hoy, Daskalakis, & Fitzgerald, 2011)
F3	2-back task	Left DLPFC	A-tDCS (reference contralateral supraorbital)	(Mulquiney, Hoy, Daskalakis, & Fitzgerald, 2011)
P3-P4	1- and 2-back span task	Posterior Parietal cortex	Interaction between task and condition (RALC vs. LARC)	(Sandrini, Fertonani, Cohen, & Miniussi, 2012)
P4	Impaired working memory recognition	Left parietal cortex	C-tDCS (reference contralateral cheek)	(Berryhill, Wencil, Branch Coslett, & Olson, 2010)

Numerical abilities					
F3-F4	Arithmetic learning, Numerical automaticity	DLPFC	Left-anodal, right-cathodal (LARC)	(Iuculano & Cohen Kadosh, 2013)	
F3-F4	Arithmetic learning	DLPFC	TRNS to bilateral DLPFCs	(Snowball et al., 2013)	
P3-P4	Basic numerical skills, numerical learning	Parietal cortex	Left-anodal, right-cathodal (LARC)	(Iuculano & Cohen Kadosh, 2013)	
P3-P4	Basic numerical skills, numerical learning	Parietal cortex	Right-anodal, left-cathodal (RALC)	(Cohen Kadosh, Soskic, et al., 2010)	
P3	Mental arithmetic	Left intraparietal sulcus (IPS)	A-tDCS (reference contralateral supraorbital)	(Hauser, Rotzer, Grabner, Merillat, & Jancke, 2013)	
P3-P4	Approximate number sense (ANS)	Bilateral parietal lobes	Bilateral tRNS	(Cappelletti, et al., 2013)	
Attention					
T4/Fz-F8/Cz	Stop-signal	Right inferior	A-tDCS	(Ditye, et al.,	

task		frontal gyrus		(reference 2012)		
				contralateral supraorbital)		
P4	Flanker task	Left	posterior parietal cortex	C-tDCS to PPC (reference 2012)	(Weiss & Lavidor, 2012)	
Executive planning						
F3	Tower of London task	Left DLPFC		A-tDCS (reference 2009)	(Dockery, Hueckel-Weng, Birbaumer, & Plewnia, 2009)	
Intelligence						
F3	Logical reasoning	Left frontal (MFG)	middle gyrus	5Hz tACS (reference vertex)	(Santarneccchi, et al., 2013)	
T3 (estimated location)	Logical problem solving	Right temporal lobe	anterior	A-tDCS (cathode on left anterior temporal lobe)	(Chi & Snyder, 2012)	

Table 1.1

Common electrode and referencing positions in tES research, using the international 10-20 or extended (20-20) system for EEG recording. Table from

(Krause & Cohen Kadosh, 2014). (LARC: left-anodal/right-cathodal; RALC: right-anodal/left-cathodal.)

1.5 Potential side effects and other consequences

The side effects of tES are relatively minor, especially compared to many types of medications. The common side effects in tES include skin irritation, nausea, and headaches, as well as occasional fatigue and iron taste (for a more detailed list, also see Table 1.2 and Fertonani, et al., in press; Poreisz, Boros, Antal, & Paulus, 2007). Conscientious and concerned scientists have started to call for formal, international regulations of the device in order to protect people and patients from suffering any kind of potential damage. Since 1998, more than 10,000 participants underwent tES procedures, without reports of cardiorespiratory arrests, seizures, or other types of brain damage (Fregni, et al., 2014). Even at the deeper cellular level, tES has been found not to harm cells, as assessed by markers for neuronal cell death (Nitsche, Liebetanz, et al., 2003). The current use of intensity and duration is far below the threshold for any kind of tissue damage, as demonstrated in rodents (Liebetanz et al., 2009). The recommended standard parameters are therefore a maximum of two sessions a day with no more than 20-60 minutes each, a current intensity lower than 2.5mA, and the use of electrodes that have a low risk for skin burns for the administered current level (Fregni, et al., 2014). Despite the low maximum intensity (2mA) a regulated DC stimulator can administer, maldeveloped do-it-yourself tES kits or severe misuse have the potential to cause tissue damage (Liebetanz, et al., 2009). This emphasizes the need for formal regulations.

In order to reduce the risk of seizures in patients or participants with unknown, pre-existing vulnerabilities, careful screening should be applied to assess both personal and family history of seizures and other neurological or neuropsychiatric conditions. Furthermore, it is important to exclude potential tES receivers who use medication and/or drugs. In particular drugs or medicine known to affect neurotransmitter levels should be avoided. A lack of sleep can also affect levels of GABA and glutamate and thereby facilitate the potential for seizures (Huber et al., 2013).

Besides the physical consequences, it is also possible to unintentionally impair cognitive functions. Growing evidence suggests that enhancement of certain cognitive functions under tES comes along with impairments in other functions (e.g. Cohen Kadosh, Soskic, et al., 2010; Iuculano & Cohen Kadosh, 2013; Rutsche, Hauser, Jancke, & Grabner, 2015; Sarkar, Dowker, & Cohen Kadosh, 2014). I will discuss these in more detail later. It is possible that the enhanced capacity in one area draws from the capacity in a proximal area, or in an area that is at least functionally connected with the stimulated one, as such areas show changes in functional connectivity after tES (Keeser et al., 2011; Zheng, Alsop, & Schlaug, 2011). In order to avoid the potential for a reversal of positive effects found at low current intensities (Moliadze, Atalay, Antal, & Paulus, 2012), I used a low current of no more than 1mA in the current thesis work. I screened participants carefully for a personal history - or family history in the case of seizures – of neurological or psychiatric conditions, alcohol or substance intake, a minimum amount of six hours of sleep in the night(s) prior to stimulation, and ensured that participants had had at least one whole meal on the day of the stimulation.

	Short-term effects	Long-term effects	Potential short-term effects	Potential long-term effects
Physical tolerability	Tingling, itching, burning sensation, pain, skin irritation (redness), headaches, fatigue (Poreisz et al., 2007)	None reported	Induction of seizures	Neurological impairments and/or risk for epilepsy
Cognitive	Task-specific	Persistence of	Maladaptation	Irreversible

effects associated with stimulated brain region	improvements or reductions in performance	improvements on experimental task (up to 6 months: Cohen Kadosh et al., 2010; Reis et al., 2009)	and dysfunctional integration of neural network under development	shaping of the network leading to faulty cognitive functioning, or transfer effects to another cognitive domain
Cognitive effects associated with other brain regions	Unknown	Unknown	Remote effects or secondary plastic changes (e.g. by lateral inhibition of the stimulated region) (Zheng, et al., 2011)	Unintended cognitive impairments compromised by dominant stimulated brain region

Table 1.2

Possible side effects in tES. Since the child brain is still in the developmental phase, induced changes are here less predictable and should be anticipated with great care. For a more detailed discussion on the topic, see (Krause & Cohen Kadosh, 2013; Krause, et al., 2013).

1.6 Excitation/inhibition and learning under tES

Learning and experience-dependent plasticity are strongly driven by the brain's major excitatory and inhibitory neurotransmitters, glutamate and GABA (gamma-aminobutyric acid), respectively (Ge & Dani, 2005; Trepel & Racine,

2000). Long-term potentiation (LTP) refers to the prolonged and enhanced activity between neurons that fire together in a Hebbian fashion. This was also described as “a lasting enhancement of synaptic transmission following high-frequency electrical stimulation” (Trepel & Racine, 1998, p. 1047). This process is therefore crucial for information storage and activity-dependent and learning-related neuroplastic changes (Turrigiano & Nelson, 2000). LTP can only occur when neurons receive sufficient activation, which is modulated by the excitatory neurotransmitter glutamate and in particular, its receptor subtype N-methyl-D-aspartate (NMDA) (Collingridge & Bliss, 1987). Reductions of GABAergic inhibition can also lead to LTP, and thereby reorganize cortical connections and even whole cortical maps indirectly (Hess & Donoghue, 1994). Furthermore, decreased GABAergic inhibition is accompanied by a facilitation in practice-based learning, whereas an increase in GABA is associated with reduced learning effects (Floyer-Lea, Wylezinska, Kincses, & Matthews, 2006; Ziemann, Muellbacher, Hallett, & Cohen, 2001). Therefore, GABAergic inhibition is viewed as a gate-keeper for LTP and activity-dependent plasticity processes (Hess & Donoghue, 1994). In theory, higher levels of glutamate and lower levels of GABA are beneficial for learning. However, a healthy brain requires a balanced interaction between excitatory and inhibitory neurotransmission to produce meaningful output and performance (Turrigiano & Nelson, 2000). For instance, overinhibition of the brain prevents LTP and reduces the output of neurons (McDonnell, Orekhov, & Ziemann, 2007). Hyperexcitability of the cortex on the contrary can cause excitotoxicity (toxic responses to excessive excitation) and therefore neuronal death (Belousov, 2012; Faden, Demediuk, Panter, & Vink, 1989).

TES has the potential to modulate brain activity and some evidence has shown that it can also modulate regional glutamate and GABA levels (e.g. Clark, Coffman, Trumbo, & Gasparovic, 2011; Foerster, et al., 2015; Polania, Paulus, Antal, & Nitsche, 2011; for a review see Hunter, Coffman, Trumbo, & Clark, 2013). The change in learning also varies with the degree of change in GABA (Stagg, Bachtiar, & Johansen-Berg, 2011). It has been suggested that the mechanism underlying tES is based on the glutamate receptor NMDA, which, as mentioned above, is also involved in LTP (Collingridge & Bliss, 1987; Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche, Fricke, et al.,

2003). TES can therefore be used to support the restoration and plastic reorganization of the brain. Considering GABA as a gate-keeper for learning-related processes, tES is thought to be able to remove the inhibitory restraints of the cortex to modulate existing levels of glutamate and GABA to rebalance the available interactions. In the following, I will describe some of the methodological and parameter options that have been explored in tES research on cognitive training.

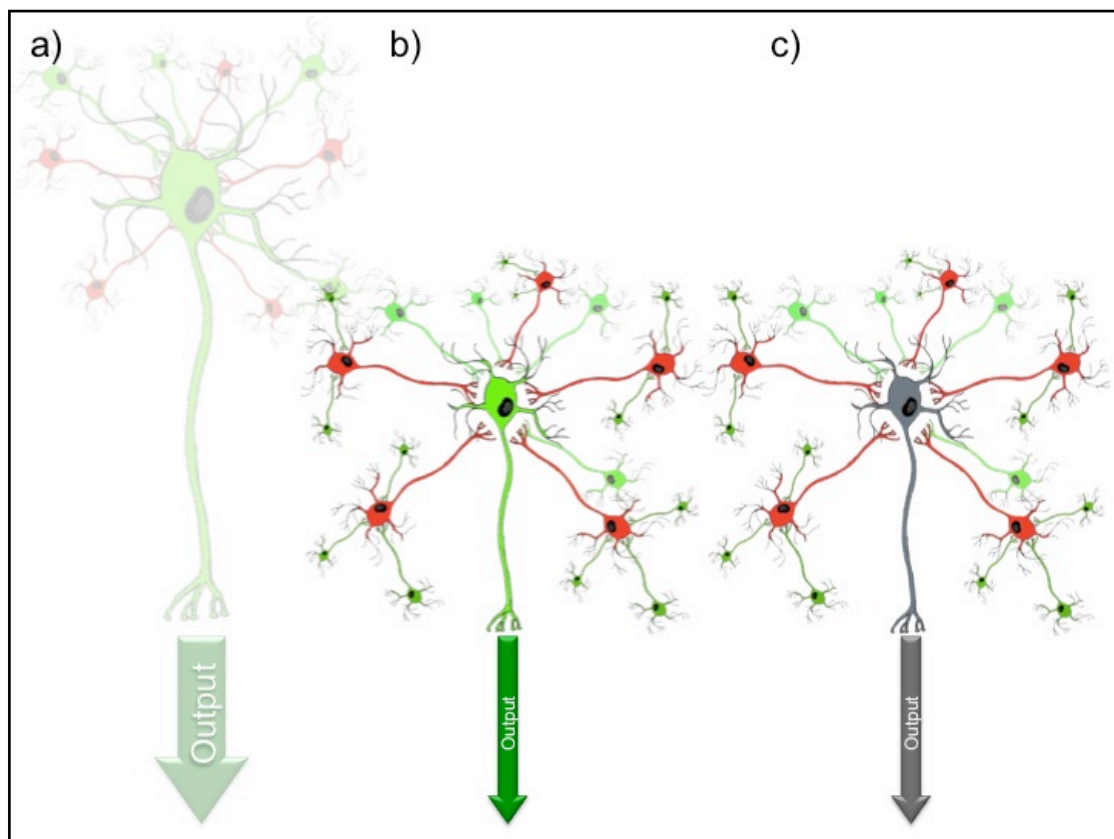


Figure 1.3

Neuronal interactions between inhibition and excitation (digitalized hand drawing of neurons). a) A balanced interaction between inhibitory and excitatory neurons on a main neuron leads to optimal output. b) Increased strength of inhibitory interneurons reduces the output of the main neuron. Not only does the output of the main neuron depend on the strength of the incoming inhibitory signals, but also on the excitatory influence of neurons feeding into the inhibitory interneurons (dark green). c) With prolonged

overexcitation, the main neuron becomes overinhibited and eventually loses its functionality.

1.7 Evidence from tES in numbers and arithmetic

The first study using anodal and cathodal tDCS configurations applied to the parietal lobes (P3-P4) in numerical cognition found beneficial effects of right-anodal, left-cathodal (RALC) tDCS on a numerical Stroop and number-space mapping task after 6 days of learning to associate artificial symbols to given magnitudes (Cohen Kadosh, et al., 2010). The researchers followed up on the results of the same sample 6 months after the study and found that the improvements were still persistent. It is important to note however, that the opposite configuration (left-anodal, right-cathodal) LARC, reduced performance on the same tasks. Similarly, 5 days of complex arithmetic training led to significant improvements in performance under tRNS to bilateral dorsolateral prefrontal cortices (DLPFCs; F3-F4), but not parietal cortices (P3-P4), which also lasted for 6 months (Snowball, et al., 2013). The same group has recently demonstrated that even physiological stress responses in form of salivary cortisol levels can be reduced and calculation times accelerated by stimulation of bilateral DLPFCs (LARC to F3-F4) of individuals with high mathematics anxiety (Sarkar, et al., 2014). The same study also demonstrated that identical stimulation impaired calculation times in individuals with low mathematics anxiety and in those participants, tDCS did not lower cortisol levels. Furthermore, both high and low mathematics anxiety participants were impaired on an additional task of executive control. In another study using a number-symbol learning over a period of 6 days, tDCS to parietal cortices (LARC to P3-P4) facilitated learning, while impairing automaticity in the testing, whereas stimulating DLPFCs (F3-F4; RALC) impaired learning and facilitated automaticity (Iuculano & Cohen Kadosh, 2013). In a tRNS (0-250Hz) study involving bilateral parietal (P3-P4), sham, an active control (tRNS but no task), and a control stimulation site (motor cortex: C3-C4), participants were trained on a number acuity task (discriminating the quantities of two differently colored clouds of dots on the screen) for 5 days and tested up until 16 weeks after. The parietal tRNS group

performed significantly better than the other conditions, which was also persistent at the follow up. Notably, the active control did not show any improvements on the pre- and post-testing tasks (Cappelletti, et al., 2013), supporting the idea that the stimulation needs to be paired with training in order to achieve positive outcomes.

All the aforementioned studies involved bilateral electrode configurations. A different research group stimulated either left, right, or bilateral parietal cortices (P3 and/or P4, electrode size 35cm²), whilst attaching the reference electrode (100cm²) to the contralateral supraorbital region (Hauser, et al., 2013). In a single-session experiment, participants were engaged in a number comparison or a subtraction task. In the former task, LARC led to more accurate number comparison performance and faster reaction times in subtraction. A study using 2mA of tPCS (to bilateral ear lobes) at random frequencies between 1 and 5Hz, found a modest facilitatory effect on complex mathematical performance (subtraction) (Morales-Quezada, et al., 2014). The effect was only found for difficult, but not easy calculation problems, and were specific to the tPCS and not the sham group. However, the effects in this study were rather weak and should be evaluated with caution. In a recent study, Rutsche et al. applied 1.5mA A-tDCS to the left posterior parietal cortex, while participants solved small (e.g. 3+4) and large (e.g. 13+27) arithmetic problems (Rutsche, et al., 2015). Participants performed the large problems faster compared to sham, but were slower on the small problems. They concluded that the role of the left PPC is different on small and large arithmetic problems. This study demonstrates the complexity of the mathematical network and how small differences in the research design can lead to large differences in the results.

Reference	Type of tES	Intensity	Target area	Improved	Impaired
(Cohen Kadosh, et al., 2010)	tDCS	1mA (20 mins)	Bilateral PPCs (P3-P4)	RALC improved artificial number	LARC reduced performance

	learning and transfer							
(Snowball, et al., 2013)	tRNS (101-640Hz)	1mA (20 mins)	(20)	Bilateral DLPFCs (F3-F4) / PPCs (P3-P4)	DLPFC stimulation improved complex arithmetic training	(PPC change)	no	
(Sarkar, et al., 2014)	tDCS (LARC)	1mA (30 mins)	(30)	Bilateral DLPFCs (F3-F4)	Decreased cortisol, improved calculation times	None		
(Iuculano & Cohen Kadosh, 2013)	tDCS (LARC)	1mA (20 mins)	(20)	Bilateral PPCs (P3-P4) / DLPFCs (F3-F4)	LARC to DLPFCs improved automaticity after learning, LARC to PPCs improved learning	LARC to PPCs impaired automaticity after learning, LARC to DLPFCs impaired learning		
(Hauser, et al., 2013)	tDCS (LARC)	1mA (25 mins)	(25)	Unilateral or bilateral PPCs (P3 and/or P4)	LARC improved number comparison accuracy and subtraction response times	None		
(Cappelletti, et al., 2013)	tRNS (0-	1mA (20 mins)	(20)	Bilateral PPCs	Number acuity training	None		

	250Hz)	(P3-P4)	(quantity discrimination) and additionally transfer to time and space discrimination		
(Morales-Quezada, et al., 2014)	tPCS (1-5Hz)	2mA (20 mins)	Earlobes	Subtraction improved on difficult, but not easy problems	None
(Rutsche, et al., 2015)	A-tDCS	1.5mA (30 mins)	Left PPC (P3)	Improved response times on large arithmetic problems	Impaired response times on small arithmetic problems

Table 1.3

Studies investigating the effects of tES on numerical or mathematical abilities. Except for the study using pulsed current (tPCS), all studies stimulated either PPC or DLPFC, some even both within the same experiment. The stimulation intensity varies between 1 and 2 mA and the duration between 20 and 30 minutes. TDCS studies occasionally compare different polarities and Cappelletti et al. even used different control conditions.

1.8 Magnetic resonance spectroscopy

Magnetic resonance spectroscopy (MRS) uses an ultra high field MRI scanner (3T or higher) to obtain a spectral quantification of typically up to thirty different chemicals in the brain. Previously known as nuclear magnetic resonance imaging (NMR), the name was changed due to the negative connotation of the expression 'nuclear' (often associated with radiation).

Unlike positron emission tomography (PET) and single-photon emission computed tomography (SPECT), MRS is non-invasive and does not require the injection of radioactive tracers. Where typical MRI measures the proton spin frequencies resulting from protons in different chemical environments (i.e. brain tissues, such as gray matter (GM), white matter (WM) or cerebrospinal fluid (CSF)), MRS distinguishes subtler frequencies of protons in different chemical environments *within* brain tissues (i.e. different metabolites). While the acquisition of a large number of voxels from the whole brain is possible for a range of neurochemicals (multi-voxel spectroscopy; MVS), the assessment of glutamate and GABA is currently only feasible using single-voxel MRS (SVS). Here a cube-shaped region of interest (the voxel of interest, VOI) is manually placed in the area of interest on the three-dimensional reconstruction of a T1-weighted scan, while the participant is in the scanner. Using sagittal, coronal and axial slices of the acquired structural scan, the desired measurement region can be selected relatively accurately. Smaller slice thickness (e.g. 1mm thick slices) provides more detailed anatomical information for the placement, however, 3mm thickness provides sufficient visualization of anatomical landmarks that are important to consider or avoid (e.g. neighboring cortical structures of CSF filled space, which induces unwanted noise in the signal). Selective pulses across the x-, y-, and z-plane excite the protons in the VOI only. Using certain acquisition techniques or sequences, a slightly larger shim volume (e.g. a recommended 3x3x3cm shim box for a voxel size of 2x2x2cm) surrounds the voxel for optimal shimming of the selected region and avoiding contamination from signals from outside the VOI. In addition, saturation bands are applied to border on the shim-volume surrounding the voxel, such that the signal originating from around the voxel can be eliminated. This process is called outer-volume suppression (OVS). (For a general introduction to the physics and analysis of MRS techniques, see Bertholdo, Watcharakorn, & Castillo, 2013; Drost, Riddle, & Clarke, 2002).

The human brain consists mostly of water, which causes a large hydrogen peak against which the metabolite spectrum scales too small to attain reliable metabolite concentrations from. In order to enhance the sensitivity towards the chemicals of interest, the water signal can be suppressed during scan

acquisition. A 1-minute sequence, the variable pulse power and optimized relaxation delays (VAPOR), achieves this.

In the work presented in this thesis, voxel spectra were acquired over the course of 8-10 minutes. 128 average spectra were recorded from 32 channels of the head coil surrounding the head. These 128 separate spectra are then all averaged together and processed. It is important to note that the signal is not acquired in slices but from the whole volume at once, therefore we acquire a single number representing the volume concentration of the neurochemical. Due to the low concentrations of metabolites, larger voxel volumes lead to higher signal-to-noise ratios (SNR) and therefore volumes smaller than 2x2x2cm are not recommended.

It is important to note that MRS is relative, rather than an absolute quantitation method. Because of the large volume of the voxel, the absolute concentration of a metabolite may be misleading. This can be due to anatomical differences between participants, but also due to biases in placing the voxel in more or less concentrated regions containing the metabolite. In order to correct for such potential biases, a reference metabolite acquired within the same VOI is used. Glutamate and GABA are most commonly referenced to creatine, which is a marker of neuronal health and metabolism, which is an optimal referencing technique for both inter- and intra-individual reproducibility (Bogner et al., 2010). At the data processing stage, it is also important to control for unequal tissue distribution across different voxels and participants. This largely controls for individual differences in anatomy and tissue constitution. For instance, MRS is intended to measure the concentration of a neurochemical *independent* of how thick or spread out the individual's cortex is within the VOI. The largest part of MRS studies include a correction for GM, WM and CSF when reporting GABA and glutamate. The concentrations of these metabolites differ across brain tissues (Choi, Lee, Merkle, & Shen, 2006).

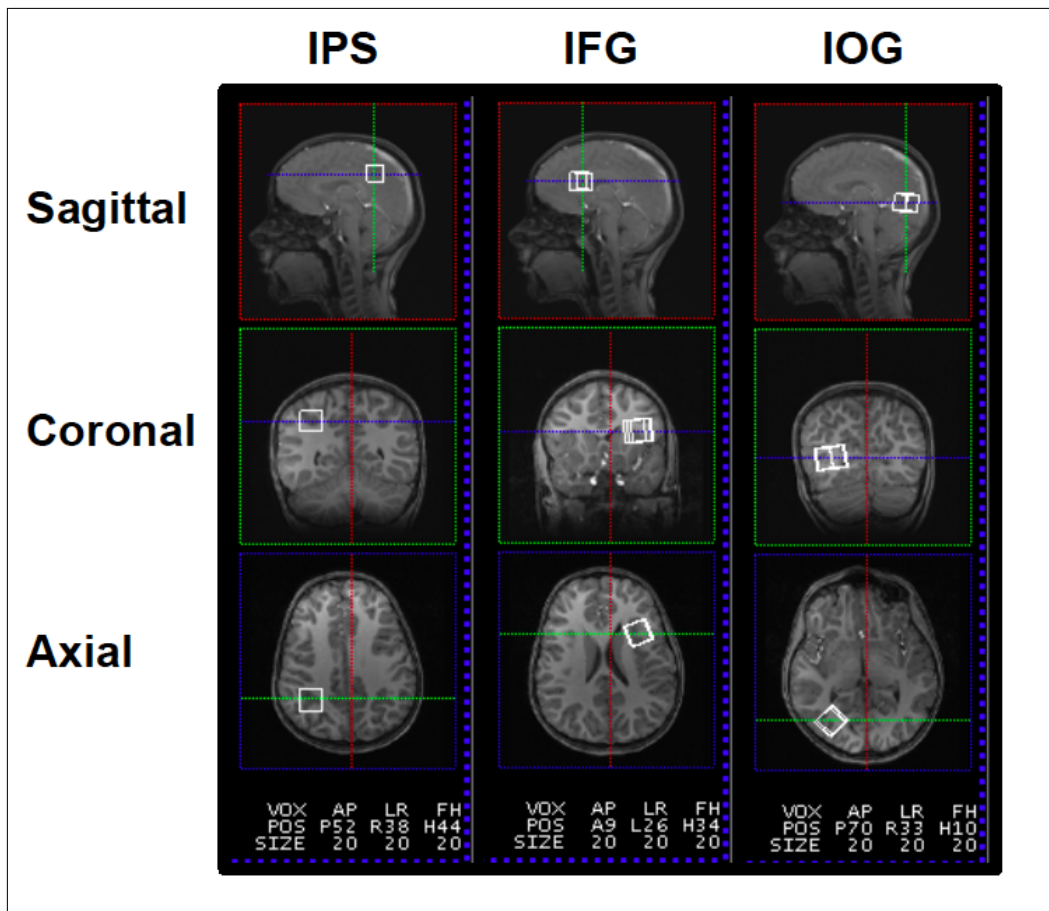


Figure 1.4

Voxel placements for three different VOIs: right intraparietal sulcus (rIPS), left inferior frontal gyrus (lIFG) and right inferior occipital gyrus (rIOG) (left to right) on sagittal, coronal and axial planes (top to bottom). Voxel positions are given in scanner coordinates (center= 0 0 0): AP (anterior-posterior), LR (left-right) and FH (foot-head). Differences in regional grey matter distributions show that the voxel is furthest away from the ventricles in the IPS VOI, and squeezed in more tightly between the cortical edge and the ventricles in the IFG and the IOG voxels in this participant. This leads to inhomogeneities of signal quality and reliability.

1.8.1 The choice of the MRS acquisition sequence

The sequence used in the present work is the spin-echo full-intensity acquired localized (SPECIAL) and has a particularly short TE (echo time; 8.5ms). Therefore it can separate glutamate from its close neighbor in spin frequency: GABA, but also from glutamine. Both neurotransmitters, but especially GABA,

occur in very low quantities and both have several peaks (GABA: 3.0, 2.3 and 1.9ppm; glutamate: 3.8, 2.3 and 2.1ppm). Due to the similarity in spin frequencies, these peaks partly overlap with each other, but also with other neurochemicals, such as creatine (Cr; 3.069 and 3.96ppm) and N-acetyl aspartate (NAA; single peak at 2ppm), which is available in larger quantities. Such overlap makes a reliable detection of the individual neurochemical technically challenging. The acquisition of the relatively weak GABA signal is therefore comparable to finding the needle in the haystack and SPECIAL is a recent development to address this problem.

Older sequences, such as MEGA-PRESS (MEscher-GARwood Point RESolved Spectroscopy) uncouple GABA from its overlaps with other chemicals (known as J-coupling) by applying different editing pulses and by subsequently using computations to subtract the signals from each other (Mullins et al., 2014). The disadvantage of this technique is that the resulting GABA signal is not measured directly (i.e. retrospective editing is required). However, if GABA measurements are of main interest, MEGA-PRESS may be considered a more optimal technique, but less so for glutamate measures (Henry, Lauriat, Shanahan, Renshaw, & Jensen, 2011). For example, the glutamate signal is acquired in combination with the signal for glutamine. This combined signal is known as glx.

One advantage of SPECIAL is its potential to separate the signal of glutamate from glutamine and providing a cleaner direct measure of glutamate. For instance, the variation in glutamine can be up to four times as high as the variation in glutamate, depending on the brain area (Kaiser, Schuff, Cashdollar, & Weiner, 2005). Therefore it is desirable to acquire the signal from glutamate without the influence of glutamine. With the newer adjustments of SPECIAL, the reliability of the measurement is greatly improved for GABA. The Cramer-Rao lower-bound (CRLB) is a measure of uncertainty of variance of each measured metabolite in a spectrum containing noise. CRLB was defined as “the lowest possible standard deviations of all unbiased model parameter estimates obtained from the data” (Cavassila, Deval, Huegen, van Ormondt, & Graveron-Demilly, 2001, see page 278). If this value is below 20% (a rule of thumb for most acquisition techniques), this corresponds to a 95% confidence interval to indicate that the acquired signal

reflects the real metabolite concentration. If the CRLB exceeds this cut-off value (i.e. >20%), there is reason to believe that the associated measurement is unreliable. Each neurochemical has its own CRLB value, and therefore the concentration in glutamate can be reliable, while the concentration in GABA may not be. Generally, GABA has the most problematic CRLBs, while glutamate and creatine are only rarely affected².

In SPECIAL, CRLBs of all acquired neurochemicals, including GABA, can be below 20% and the line widths are narrow (Mekle et al., 2009). Furthermore, SPECIAL has good sensitivity and localization performance of the voxel magnetization, and a decent signal-to-noise ratio (SNR) to allow for the reliable quantitation of various neurochemicals. It is important to note, however, that such short-TE studies only acquire reliably, when certain experimental conditions, i.e. good line width and SNR are warranted (Near et al., 2013). (For a general discussion on common artifacts in MRS, see (Kreis, 2004). The reliability of the measurements is commonly established and validated by scanning phantoms. A phantom is a bottle or other vessel containing a known concentration of the compounds in question, and comparing the measured with the real known concentration (Henry, et al., 2011; Mekle, et al., 2009; Near, et al., 2013).

² This applies to the neurochemicals used in this thesis. I reported measures and strategies of quality control in the chapters containing MRS data.

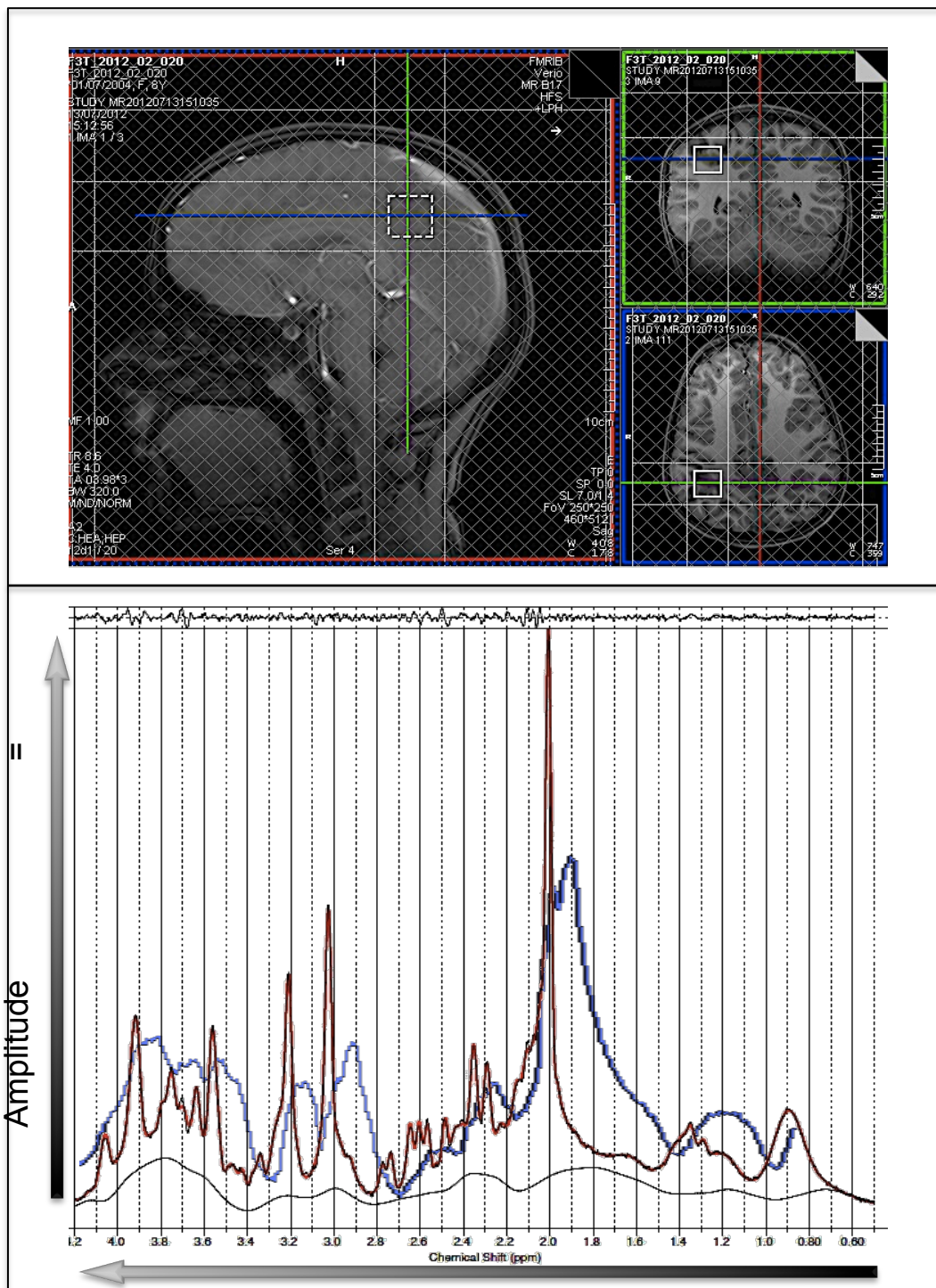


Figure 1.5

3D view of voxel positioning on a reconstructed T1-weighted scan in the right IPS in a child during the scan (upper panel). The lower panel shows the unprocessed spectrum (blue line) of the same voxel in this individual overlaid on top of the processed spectrum (red line) after frequency drift, baseline, and phase corrections. Note how the initially noisy signal now shows more defined

peaks. Narrow line width (expressed in ppm or Hz) refers to narrow peaks allowing accurate measurement with little uncertainty. The lower black line represents the baseline and the top bar shows the noise subtracted from the spectrum. The concentration of the metabolites is measured by the amplitude of the peaks (y-axis) and the proton spin frequency is mapped along the reverse x-axis and represented by parts per million (ppm).

1.8.2 The practical use of glutamate and GABA measurements

Magnetic resonance spectroscopy (MRS) has gained in popularity over the past 10-15 years, due to its enhanced ability to distinguish nervous system disorders from healthy brains at the neurochemical level non-invasively. The most useful neurochemicals for such potential diagnostic purposes are creatine (Cr), myo-inositol (Myo), n-acetyl aspartate (NAA), choline (Cho) and lactate. With up to 70 different human neurochemicals, even older applications of MRS have been suggested as useful diagnostic measurement technique to identify neurological disorders, including Alzheimer's disease (AD), brain tumors, developmental disorders, head trauma, and stroke (Tran, Ross, & Lin, 2009). Similarly, with the advent of more recent signal acquisition sequences, scientists have started to investigate glutamate and GABA in order to identify the diseased brain based on group-level differences. GABA can distinguish healthy brains from Alzheimer's patients (Bai et al., 2014), fibromyalgia and other types of chronic pain (Foerster et al., 2012; Harris & Clauw, 2012), and autism (Kubas et al., 2012; Rojas, Singel, Steinmetz, Hepburn, & Brown, 2013). Due to the involvement of cognitive abnormalities in these disorders, scientists have discovered the opportunity to study more subtle cognitive problems using quantitations of GABA and glutamate, such as in schizophrenia (Goto et al., 2009; Rowland et al., 2012; Tayoshi et al., 2010; Yoon et al., 2010) attention-deficit hyperactivity disorder (ADHD) (Arcos-Burgos et al., 2012; Carrey, MacMaster, Gaudet, & Schmidt, 2007; Edden, Crocetti, Zhu, Gilbert, & Mostofsky, 2012), and panic disorder (Long et al., 2013). Since functional magnetic resonance imaging (fMRI) is thought to measure 'neuronal mass activity' (Logothetis, 2008, p. 870), this measure of metabolites potentially provides a more sensitive indicator of brain mechanisms. Larger groups of neurons may not necessarily be damaged (as

measured by markers of neuronal health), but may exhibit atypical levels of excitation or inhibition, thereby preventing efficient and meaningful cognitive processing.

Recent MRS findings have demonstrated that human performance can also be related with glutamate and GABA levels and these can be artificially modulated with learning (Floyer-Lea, et al., 2006; Sampaio-Baptista, et al., 2015). This demonstrates that despite the low spatial resolution, MRS can be used to track learning-related changes at a deeper biological level than conventional MRI methods. For example, 30 minutes of motor sequence learning can already reduce GABA levels in the primary motor cortex by approximately 20% in an 8ml cubic voxel (MEGA-PRESS) (Floyer-Lea, et al., 2006). This reduction was specific to the learning, but not a control task. In combination with tDCS applied in between two scans, glx has been shown to increase after 30 minutes of anodal tDCS (2mA, electrode size 11cm², without a learning task) to the right intraparietal sulcus (IPS) (with the cathode on the left arm) in a right but not left IPS voxel (cubic 8ml voxel) (Clark, et al., 2011).

1.9 Research questions

In the present work, I investigated how regional, noninvasive measures of glutamate and GABA in some of the most prominent areas of the brain mathematical network were related to numerical and arithmetic abilities. Additionally, I investigated this link in children and adults. Furthermore, I used tES to frontal and parietal cortices in order to investigate the effects on arithmetic abilities and neurochemicals. I used stimulation protocols that have been proven successful in the past. Based on the scientific literature on tDCS, tRNS, MRS and arithmetic training, I aimed to answer the following questions:

1. Can previous results from our lab on the effect of tRNS on arithmetic training be replicated using a different study design (see chapter 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation')?
2. Are glutamate and GABA in brain areas involved in mathematical abilities associated with cognitive measures of mathematics? Are they only associated separately or is the ratio of glutamate and GABA

associated with mathematics? Are potential associations different in children and adults due to developmental changes in the brain (see 'Inhibition, excitation, and cognitive achievement in the developing brain', and 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation')?

3. Are glutamate and GABA concentrations in brain areas associated with mathematics distinct in an individual with exceptional calculation abilities (see chapter 'The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance')?
4. Is it possible to boost calculation performance of an individual with already exceptional abilities using excitatory tES? Can the performance of expert calculators who are not prodigies be further improved by excitatory tES (see chapter 'Neuroenhancement of High-Level Cognition: Pushing the Boundaries of the Human Brain')?
5. Can glutamate and GABA concentrations be modulated using excitatory tES during arithmetic training (see chapter 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation')?

2 Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation

Abstract

My lab previously demonstrated that five days of transcranial random noise stimulation (tRNS) to bilateral dorsolateral prefrontal (DLPFCs), but surprisingly not posterior parietal cortices (PPCs), led to improvements in arithmetic learning and facilitated brain metabolism compared to sham stimulation. Due to the role of the posterior parietal cortex in numerical cognition and Mathematics, I replicated the study in a double-blind, placebo-controlled, within-subject design to reduce inter-individual variation. I used 20 minutes of 1mA of high-frequency tRNS (101-640Hz) over five consecutive days of arithmetic training. I counterbalanced bilateral tRNS to DLPFCs and PPCs, as well as sham stimulation. A wash-out period of 2 weeks between stimulation conditions was added. TRNS to DLPFCs led to reduced calculation times for untrained arithmetic problems (transfer effect), whereas parietal tRNS showed higher training accuracy and longer initial response times, which could not be explained by a speed-accuracy trade-off. I demonstrate here that tRNS to bilateral DLPFCs, but also parietal cortices significantly improved arithmetic performance on transfer and training tasks, respectively, in above-average calculators.

2.1 Introduction

Transcranial electrical stimulation (tES) is currently extensively studied for its potential to improve cognitive functions, and is especially desirable for individuals with cognitive deficits or disorders associated with poor cognitive development (Krause & Cohen Kadosh, 2013; Vicario & Nitsche, 2013a, 2013b). Most studies apply the stimulation to brain areas known to be associated with the cognitive deficit and the task administered. For instance, left posterior temporal cortex stimulation with transcranial direct current stimulation (tDCS) improved word reading in low-performance readers (Turkeltaub, et al., 2012). This area is consistently associated with word reading and word reading deficits (Houde, Rossi, Lubin, & Joliot, 2010; Paulesu et al., 2001; Silani, et al., 2005), such that the choice of the stimulation region was relatively straightforward and successful.

Arithmetic abilities, similar to reading abilities, have a life-long impact on an individual's life achievement including levels of education, socioeconomic status, and future salary (Parsons & Bynner, 2005; Ritchie & Bates, 2013). Therefore, it is desirable to enhance cognitive training effects in those with arithmetic difficulties (Cohen Kadosh, et al., 2013; Stein, et al., 2011). Due to its requirement for various higher cognitive functions (e.g., quantity processing, numerical fact retrieval and working memory), arithmetic abilities involve a complex fronto-parietal brain network (Kaufmann, Kucian, & Aster, in press; Kaufmann, et al., 2011). Besides the consistent activation of a network involving DLPFCs and intraparietal sulci (IPS), Delazer et al. (Delazer et al., 2005) additionally found that memory retrieval-based numerical tasks involved bilateral precunei and the left angular gyrus (AG) more strongly than when participants had to mentally compute complex calculations. This finding was in line with the idea that during early learning stages where computation is the prominent calculation strategy, frontal regions are more involved in the task, and as the trained material becomes more familiar and automatic, parietal areas become more involved and frontal activation weaker (Zamarian, Ischebeck, & Delazer, 2009).

In a previous five-day cognitive training study, it was shown that complex arithmetic performance could be improved by enhancing excitability in

bilateral DLPFCs using transcranial random noise stimulation (tRNS). Moreover, such stimulation combined with training could facilitate metabolic activity in lateral prefrontal areas, as measured using near-infrared spectroscopy (NIRS) (Snowball et al., 2013). Surprisingly, the stimulation effect was specific to the DLPFC, as parietal tRNS had no effect on either behavioral or metabolic activity (measured at the DLPFC). The training task in this study was adopted from Delazer et al.'s study (Delazer et al., 2005), in which fronto-parietal increases in the blood-oxygenated level dependent (BOLD) response were found in trained as well as untrained arithmetic problems engaging active computation and fact retrieval strategies. The IPS is known to be involved in the processing of quantity (Cantlon, Platt, & Brannon, 2009; Cohen Kadosh, Lammertyn, & Izard, 2008) and the angular gyrus in fact retrieval (Zamarian et al., 2009), such that increases in cortical excitability in these areas may have effects on calculation performance. In previous studies polarity-specific parietal (P3-P4 electrode positions in the international 10-20 system for EEG recording) tDCS has been shown to affect numerical and arithmetic abilities (Cohen Kadosh, Soskic, et al., 2010; Hauser, et al., 2013). While those studies found differences in laterality, this could be explained by the differential involvement of the two hemispheres in the applied task materials (Stanescu-Cosson et al., 2000). Bilateral stimulation, such as tRNS, has been shown to have stronger effects than lateralized stimulation (Saiote, Polania, Rosenberger, Paulus, & Antal, 2013; Terney, et al., 2008), and also beneficial in basic numerical processing when applied to the parietal lobes (Cappelletti et al., 2013). Due to these findings, I hypothesized that optimizing the research design of our previous study would reveal beneficial effects of parietal tRNS on arithmetic training.

In order to investigate the potential of tRNS to improve complex arithmetic abilities, I replicated the previous study (Snowball et al., 2013). I used a double-blind, within-subject design stimulating DLPFCs, PPCs and sham to either region to reduce the inter-individual variability in mathematics performance. All participants were tested by the same experimenter in order to also reduce the potential influence of different experimenters. Participants were trained for five consecutive days whilst receiving 20 minutes of tRNS, then undertook a transfer task after the end of the final training session.

2.2 Methods

2.2.1 Participants

10 healthy, right-handed undergraduate students (4 female) participated in the current experiment (19-24 years old, mean age 20.9, SD=1.91 years). All had normal or corrected-to-normal vision, and none of them reported any history of neurological or psychiatric disorders. Written informed consent was acquired at the beginning of the study and potential risk factors including alcohol or drug intake on the previous day and number of hours of sleep, were assessed before each stimulation session. The study has been approved by Berkshire Research Ethics Committee.

2.2.2 Standardized mathematical assessment

The Wechsler Individual Achievement Test (WIAT[®]-II^{UK}) assesses standardized measures for basic mathematical abilities (Wechsler, 2005). This test was chosen, as it provides standardized scoring tables for both children and adults of all ages and can therefore be compared across age groups. In the subpart called 'numerical operations', increasingly difficult numerical operations are solved on the paper, while in subpart 'mathematical reasoning, the logical application of arithmetic to increasingly difficult verbally and visually presented mathematical problems have to be used. More complex examples of the numerical operations test are:

1. $\sqrt{49} = \underline{\hspace{2cm}}$
2. $3^2 + 4^2 = \underline{\hspace{2cm}}$
3. 300% of 75 =
4. $\frac{1}{4} \times \frac{1}{5} = \underline{\hspace{2cm}}$
5. $28 \div 7 - 5 = \underline{\hspace{2cm}}$

More complex examples of the mathematical reasoning subtest are:

“Sarah had £200. She bought four tires. Each tire cost forty-five pounds and fifty cents plus three pounds each for the balancing. How much does she have left?”

“How many different ways are there to arrange four books on a shelf?”

For each of the subtests, I computed the standard scores according to the Wechsler age-standardized scoring tables. A composite score can be computed from the combined standard scores of the two subtests. It is important to note that the subtests assess different aspects of abilities, which may be differently related to the tasks used in the current thesis. While the numerical operations test requires good knowledge of a variety of different problem types, mathematical reasoning requires less knowledge but more adaptive thinking. Numerical operations was always the first, while mathematical reasoning was administered afterwards. Participants received pen and paper to work out problems on the paper. *This task was used in all experimental chapters and in the exact same way.*

2.2.3 Complex arithmetic training

A five-day training paradigm was adapted from a previous fMRI study and similar to the one my lab has used previously (Delazer et al., 2005; Snowball et al., 2013; Figure 2.1). Not only does the task involve training, but in a separate session, novel and adapted combinations of arithmetic problems can be used to test whether the trained material transfers to untrained material. Furthermore, the task involves two different types of arithmetic: the use of rote memorization and the use of algorithmic strategies to compute problem solutions. The results from the fMRI study provided the landmarks for the electrode positioning in our tES studies.

The following training and transfer procedure was also used in the chapter ‘Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation’.

2.2.3.1 Training procedure

Here participants were trained to solve complex equations either by applying a fixed algorithm to a given pair of operands presented on the screen ('strategy' or 'calculation' problems, represented by '§', Fig. 1A), or by memorizing pairs of given problems and their solutions ('drill' problems, denoted by '#', Fig. 1B). Each week participants used a different one of the following strategy algorithms: " $2 * B - A + 1$ "; " $2 * (A+B) - 10$ "; and " $2 * B + A - 10$ ". With this algorithm provided and visible to the participants on day 1 only, two numbers ('A' represented the left and 'B' the right operand) were presented on the screen together with the symbol indicating 'strategy problem': '§'. The algorithm was the same for each 5-day training period. The order of the algorithms was counterbalanced across participants and training periods. Similarly, drill problems were the same for each 5-day training period, and were replaced with a new set of problems when a new 5-day training period started. This combination of three different weeks and two different operations resulted in six separate sets of operand pairings, which were randomized to balance potential differences in task difficulty across the repeated within-subjects sessions.

The number of training problems varied across the 5 days starting with 10 blocks of problems on day 1, 12 on day 2, 14 on day 3, 16 on day 4 and 14 on day 5. Each block consisted of 18 trials (3 repetitions of the 6 problems, pseudo-randomized). The duration of presentation of problems on the screen decreased each day for both strategy and drill, and the response window for drill problems decreased as well.

The task was presented on a 17" computer screen and the task duration for the training was approximately 30 minutes per day for five consecutive days. Each trial started with a blank screen (200ms), followed by a fixation cross presented for 300ms. After an initial presentation of the problem, a mask (250ms) and blank screen (250ms) followed and the problem reappeared a second time, persisting until the participant had entered the two-digit response using the keypad (numbers from 0-9). In the strategy condition, there was unlimited time for the response, whereas the drill problem had to be answered within a given interval that decreased each day. Feedback was presented for 500ms after every response, displaying either "Correct answer", "Mistake", or

“No response.” In the latter two cases, the problem was repeatedly presented until answered correctly. Strategy and drill blocks were interleaved within each training day and the presentation order was randomized and counter-balanced across participants and weeks.

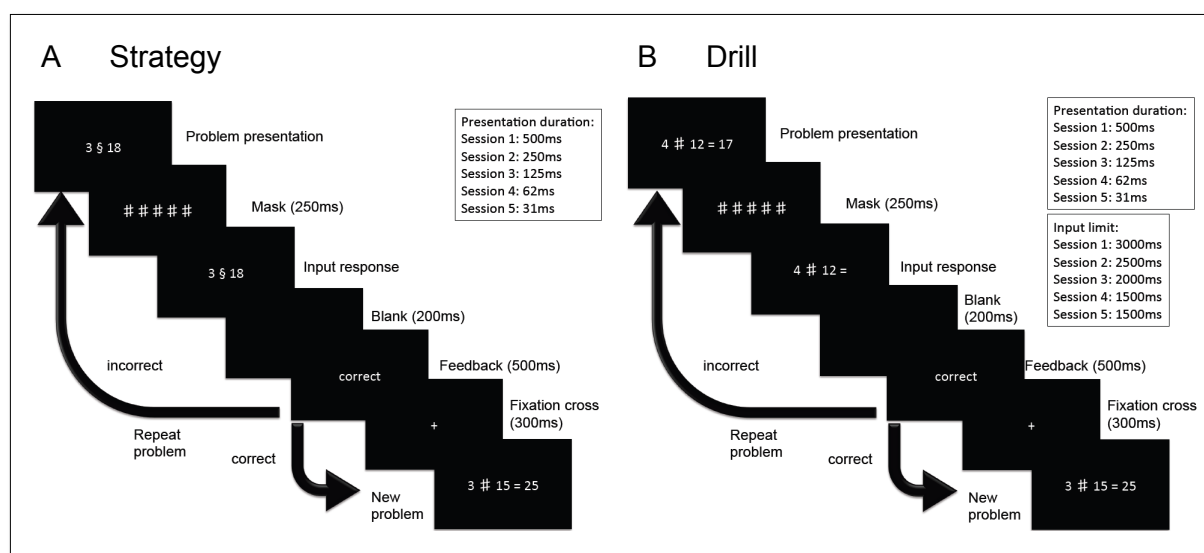


Figure 2.1

Task procedure. (A) Strategy problems (denoted as ‘§’) began with the presentation of a problem (presentation time decreased from one session to the next), a brief mask and an unlimited input duration. Upon correct response, a new problem was presented. Incorrect responses led to the repetition of the same problem until the correct response was provided. (B) Drill problems (denoted as ‘#’) were presented as a combination of two operands and an outcome without knowing the underlying algorithm to derive the result by calculation. The combination had to be memorized and reproduced after a mask. Both presentation time and response input times decreased from one session to the next. Problems were repeated when answered incorrectly.

2.2.3.2 Testing phase (transfer of trained material)

To assess for transfer effect, I administered an additional task on the fifth day after completing the training. This task included 6 trained and 6 untrained (novel) arithmetic problems for both strategy and drill, and was administered

in a similar computer task. No stimulation was administered during this period. Each problem was presented three times and unlimited response time was allowed. The untrained strategy problems investigated whether the training had conferred algorithmic calculation skills transferable to new arithmetic situations, whereas the addition of new drill problems assessed whether the participants had identified the underlying solving algorithms.

2.2.4 tRNS

High frequency tRNS (101-640Hz) was delivered using a Magstim[®] stimulation device and was applied to bilateral prefrontal (F3-F4) or parietal (P3-P4) electrode sites according to the international 10-20 system for EEG recording. 5x5cm electrodes covered by conductive synthetic rubber sponges were soaked in saline solution and applied to the scalp surface where it was held in place by rubber straps. 1mA was administered for 20 minutes starting with the beginning of the training task (or 20 seconds of corresponding sham stimulation) with 15 seconds ramp-up and ramp-down each.

2.2.5 Procedure

Each participant took part in all three stimulation conditions (DLPFC, parietal and sham to either site) with a wash-out period of two weeks in between each pair of conditions to avoid carry-over effects. The order of conditions and calculation algorithms was randomized and counter-balanced across participants. Both participant and experimenter were naïve to the stimulation condition. A safety questionnaire was filled out before each stimulation session to assess the participants' eligibility for brain stimulation on each day in particular (e.g., to exclude the possibility for alcohol intake on the previous day). Baseline measures of mathematical abilities independent of the training task were measured using the mathematical tests of the Wechsler Individual Achievement Test (WIAT-II-UK; Wechsler, 2005). On the first of five training days, participants received a sheet showing the algorithm they had to apply to the calculation problems. This sheet was removed after the first session. Participants performed 45 minutes of training with an initial 20 minutes (or 20 seconds) of tRNS on all five days, followed by a 30 minute testing procedure on the fifth day after completion of the training.

2.3 Results

The dependent variables were response times (RTs) of correctly answered problems, accuracy in percentage, and the learning rate (see Calculation learning curves across the five training days. Standard scores of mathematical reasoning (WIAT-II-UK) were entered as a covariate in all ANOVAs to control for individual differences in baseline abilities. The threshold for significance was set at an alpha level of 0.05. In cases of violation of the Mauchly's test of sphericity, Greenhouse-Geisser correction (GG) was applied. Note that in contrast to a previous study, which used a between-subjects design (Snowball et al., 2013), I used a within-subjects design that does not allow for the use of task performance on the first block as a covariate, since condition as a within-subject factor involves three different levels, which cannot be simultaneously accounted for.

Gender	Age	Numerical operations	Mathematics reasoning	Composite score	Stimulation order
Male	22	125	126	135	SFP
Male	21	121	123	128	SPF
Male	20	120	122	126	PFS
Male	21	103	117	109	PSF
Male	19	118	120	122	FPS
Male	19	126	120	130	FSP
Female	24	125	117	126	FPS
Female	19	111	120	116	SFP
Female	24	101	87	93	PSF
Female	21	123	120	127	FPS

Table 2.1

Gender, age, mathematical standard scores and stimulation order for all ten participants of the study. Note that one female has lower than average mathematical reasoning, but average numerical operations scores. She was therefore included in the study. The stimulation order was carefully counter-balanced across the sample to avoid systematic carry-over effects. The last

column shows the order in which participants received the stimulation (F=frontal, P=parietal, S=sham). Furthermore, the order in which drill and calculation algorithms were used across the three conditions was also counter-balanced across the participants.

2.3.1 Statistical power

Using G-Power 3.0, I computed the sample size required for optimal power (95%) in a repeated measures within-subject design. Assumed was a moderate effect size of .25 ($\eta_p^2=.06$) and a correlation between repeated measures of $r=.5$. When considering a single measurement (e.g. learning rate) per condition (i.e. three repeated measurements per participant), the required total sample size is $N=42$. Considering the repeated measures for the five training days (i.e. five measurements per participant), the total sample size could be reduced to $N=31$. Observed power is not reported for this or the following chapters, as the interpretation of retrospective power calculations is considered flawed (Hoenig & Heisey, 2001).

2.3.2 Training

I performed 3x5 (condition x day) factorial repeated measures ANCOVAs with mathematical reasoning as a covariate for each task (calculation and drill) separately.

2.3.2.1 Calculation response times

A 3x5 (condition x day) factorial within-subject repeated measures analysis of covariance (ANCOVA) with mathematical reasoning as a covariate was performed on the mean RTs for calculation problems and showed a significant interaction effect between condition and day ($F(1.8,14.5)=7.08$, $p=.008$, $\eta_p^2=.45$). Within-subjects contrast comparisons revealed a significant fourth order linear trend ($F(1,8)=11.83$, $r=0.83$, $p=.009$, $\eta_p^2=.6$). There was an initial decrease and a slight subsequent increase in response times towards the end of the training period, when problem presentation times became shorter and task difficulty increased. This interaction was due to faster learning under parietal tRNS. In the parietal condition, the effect of day was significant ($F(1.59,12.68)=30.13$, $p<.001$, $\eta_p^2=.79$) but not in the frontal group ($p=.17$).

Parietal stimulation showed significant improvements in RTs from day 1 compared with all other days ($p < .003$) and further from day 2 to 3 ($p = .006$) and day 2 to day 4 ($p = .002$). There was only a trend for an improvement from day 3 to 4 ($p = .07$), and a non-significant slowing from day 4 to 5 ($p = .16$). In the sham group, the effect of day was also significant ($F(2.03, 16.21) = 6.12$, $p = .001$, $\eta_p^2 = .43$). Only day 1 was significantly slower than all other days ($p < .003$), but there were no further improvements within the group ($p > .1$).

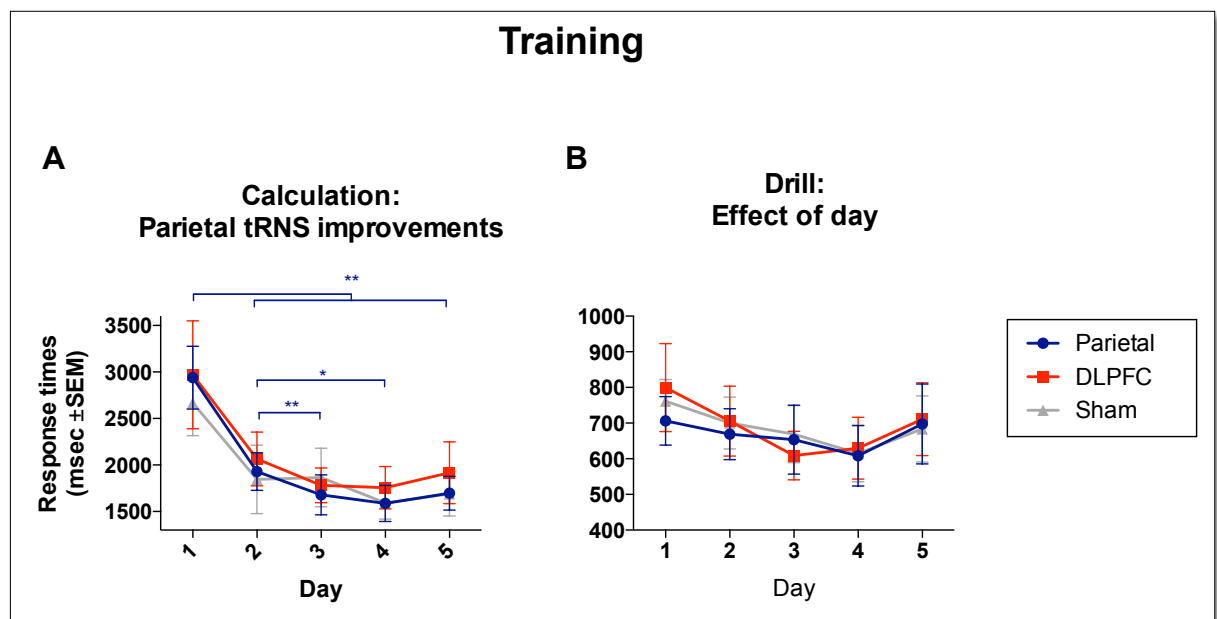


Figure 2.2

Training response times. A) Interaction between day and condition for calculation response times. The subsequent main effect of day per level of condition for calculation speed was only significant for sham and parietal tRNS. After an initial improvement from day 1 to 2, only parietal tRNS showed marked improvements across the training. B) There was no condition effect for drill ($*p < .05$, $**p < .01$).

2.3.2.2 Drill response times

The same analysis on participants' mean RTs for drill problems showed a significant main effect of day ($F(4, 32) = 4.3$, $p = .007$, $\eta_p^2 = .35$) but not condition ($F(1, 8) = .83$, $p = .45$, $\eta_p^2 = .09$).

2.3.2.3 Calculation Accuracy

A 3x5 (condition x day) factorial within-subject repeated measures analysis of covariance (ANCOVA) with mathematical reasoning as a covariate was performed on the mean accuracy (%) for correctly answered calculation problems. There was a significant main effect of condition ($F(2,16)=3.99$, $p=.04$, $\eta_p^2=.33$), which was due to higher accuracy in the parietal than the sham condition ($F(1,8)=6.27$, $p=.04$, $\eta_p^2=.44$). DLPFC and sham stimulation did not differ significantly ($F(1,8)=0.27$, $p=.62$, $\eta_p^2=.03$), while there was a trend for higher accuracy in the parietal compared to DLPFC stimulation ($F(1,8)=4.32$, $p=.07$, $\eta_p^2=.35$) (Fig. 2A).

2.3.2.4 Drill accuracy

The same analysis on participants' mean percentage accuracy for drill problems revealed no significant main (effect of day: $F(4,32)=.26$, $p=.9$, $\eta_p^2=.03$; condition: $F(2,16)=.13$, $p=.88$, $\eta_p^2=.02$) or interaction effects ($F(8,64)=.7$, $p=.7$, $\eta_p^2=.08$).

2.3.2.5 Calculation learning curves

In order to investigate the effect of tRNS on the learning increment, initial performance in terms of the response times (beta: β) and the learning rate (alpha: α) were fitted into a power law function: $RT = \beta \cdot (N)^{-\alpha}$, in which RT is the individual participant's mean calculation training response time for correctly answered items on a given day or session N. Non-linear regression analyses on the response times provided the intercept (β) and slope (α) of the (exponential) regression line. The resulting β represents the response time on the first block of the training, and α indicates the learning rate over the course of the five days of training. Repeated-measures ANCOVAs with stimulation condition as a within-subject factor and β as the dependent variable with mathematical reasoning as a covariate revealed a significant main effect of stimulation for β values ($F(2,16)=5.99$, $p=.01$, $\eta_p^2=.43$). Compared to sham, β 's were significantly higher under parietal ($F(1,8)=24.67$, $p=.001$, $\eta_p^2=.76$), but not the DLPFC condition ($F(1,8)=0.65$, $p=.44$, $\eta_p^2=.08$), and there was a marginal difference between DLPFC and parietal conditions ($F(1,8)=4.55$,

$p=.07$, $\eta_p^2=.36$). The combined effect of DLPFC and parietal conditions compared to sham was significant ($F(1,8)=8.79$, $p=.02$, $\eta_p^2=.52$) (Fig. 2B). There was a trend for an effect for α ($F(2,16)=2.77$, $p=.09$, $\eta_p^2=.26$). This was due to a significantly higher learning rate (α) in the sham compared to the parietal tRNS condition ($F(1,8)=5.55$, $p=.046$, $\eta_p^2=.41$). I did not examine the effect of stimulation on learning rate for drill problems, as it violates some of the required assumptions for such analysis (Snowball et al., 2013).

2.3.3 Transfer

2.3.3.1 Calculation accuracy

A 3 (stimulation condition) x 2 (trained vs. untrained problems) factorial repeated-measures ANCOVA with mathematical reasoning as a covariate was performed on participants' mean percentage accuracy for calculation problems and revealed no significant main (condition: ($F(2,16)=0.46$, $p=.96$, $\eta_p^2=.006$); trained vs untrained: $F(1,8)=.47$, $p=.51$, $\eta_p^2=.06$) or interaction effect ($F(2,16)=0.05$, $p=.95$, $\eta_p^2=.006$). The lack of effects might be attributed to a ceiling effect for trained problems, with only slightly lower performance on untrained problems (Fig. 2C).

2.3.3.2 Calculation response times

The covariate of mathematical reasoning was significant ($F(1,8)=8.76$, $p=.02$, $\eta_p^2=.52$). Moreover, I found a significant interaction between trained versus untrained calculation problems and stimulation condition ($F(2,16)=9.77$, $p=.002$, $\eta_p^2=.55$). Subsequent one-way repeated-measures ANCOVAs with mathematical reasoning as a covariate were performed for trained and untrained problems separately and revealed a significant main effect of stimulation condition for untrained problems ($F(2,16)=15.43$, $p< 0.001$, $\eta_p^2=.66$) but not for trained problems ($F(2,16)=0.05$, $p=.95$, $\eta_p^2=.006$). Within-subjects contrasts of the untrained problems showed faster response times for DLPFC compared to the sham condition ($F(1,8)=13.52$, $p=.006$, $\eta_p^2=0.63$) and to the parietal condition ($F(1,8)=32.26$, $p<0.001$, $\eta_p^2=0.80$). Parietal and sham conditions did not differ significantly from each other ($F(1,8)=1.0$, $p=.35$, $\eta_p^2=.11$).

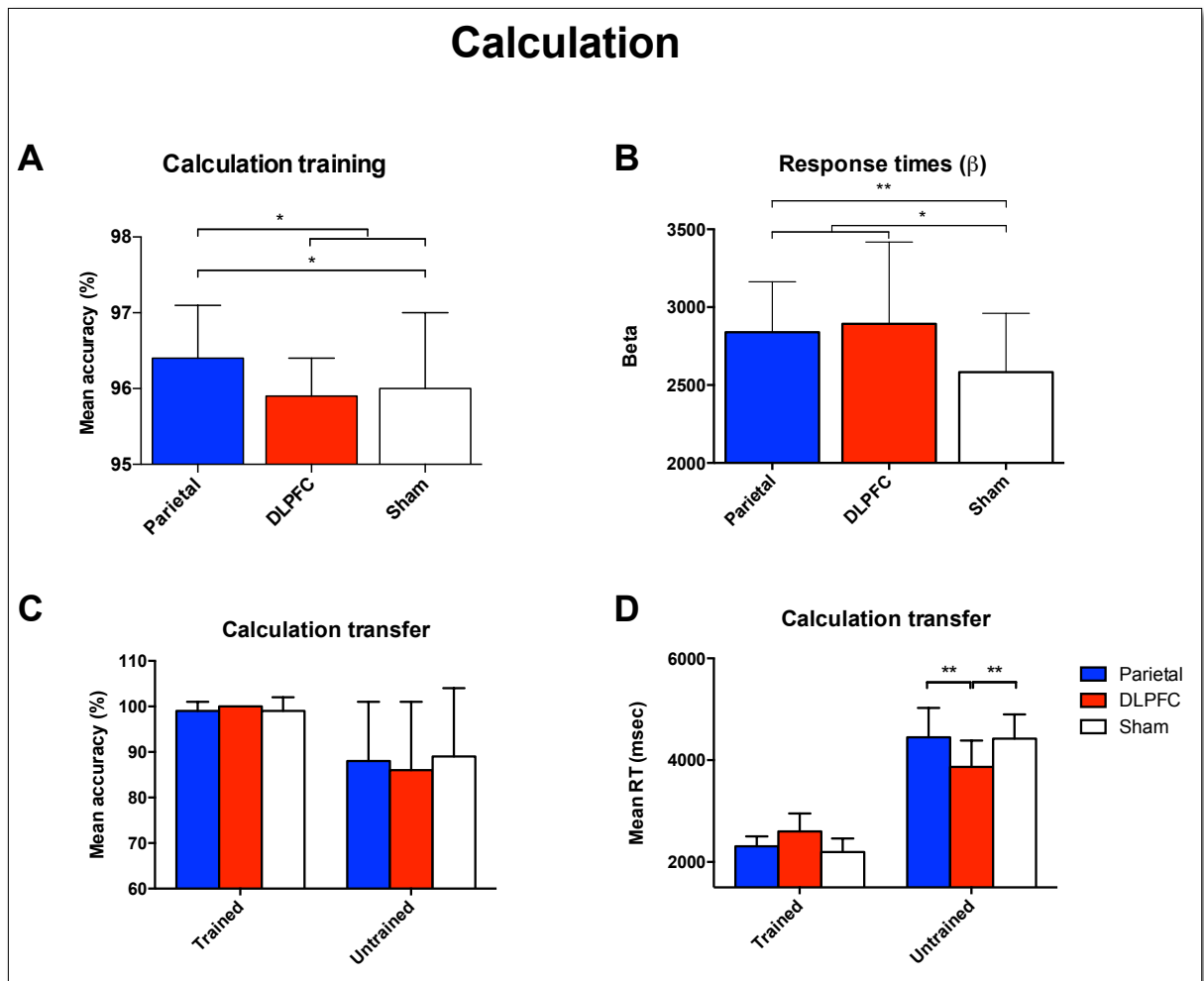


Figure 2.3

Training and transfer for calculation problems. (A) While DLPFC and sham conditions did not differ in their training accuracy, parietal stimulation showed significantly higher accuracy compared to sham, as well as compared to sham and DLPFC combined ($p < .05$). (B) The parietal group showed a significantly slower initial performance compared to sham, as estimated by the non-linear regression analysis (beta parameter). (C) Mean accuracy on the trained problems was almost 100% (ceiling effect), whereas the accuracy for untrained problems was slightly lower (~90%). There was no significant main effect of stimulation group, or interaction between stimulation and trained/untrained problems ($p > .09$). (D) DLPFC tRNS showed the fastest performance on the transfer task. The DLPFC group was significantly faster than the parietal and sham groups on untrained problems ($p < .01$).

2.3.4 Drill accuracy

Performance for untrained drill questions was poor (participants did not know the underlying algorithm to derive the result), leading to a complete lack of correct answers for some participants and therefore missing values in the data analysis (accuracy in %, and standard deviations: trained: DLPFC=87% (27%), parietal=85% (28%), sham=71% (14%); untrained: DLPFC=6% (6%), parietal=13% (20%), sham=9% (8%)). Therefore, only participants' performance on trained drill problems will be reported. The main effect of a repeated-measures ANCOVA on mean accuracy for trained drill problems with mathematical reasoning as a covariate showed no significant differences between stimulation types ($p>.45$) (Figure 2.4).

2.3.5 Drill response times

There was no significant effect of stimulation for drill response times on trained problems ($F(2,16)=2.86$, $p=.09$, $\eta_p^2=.26$).

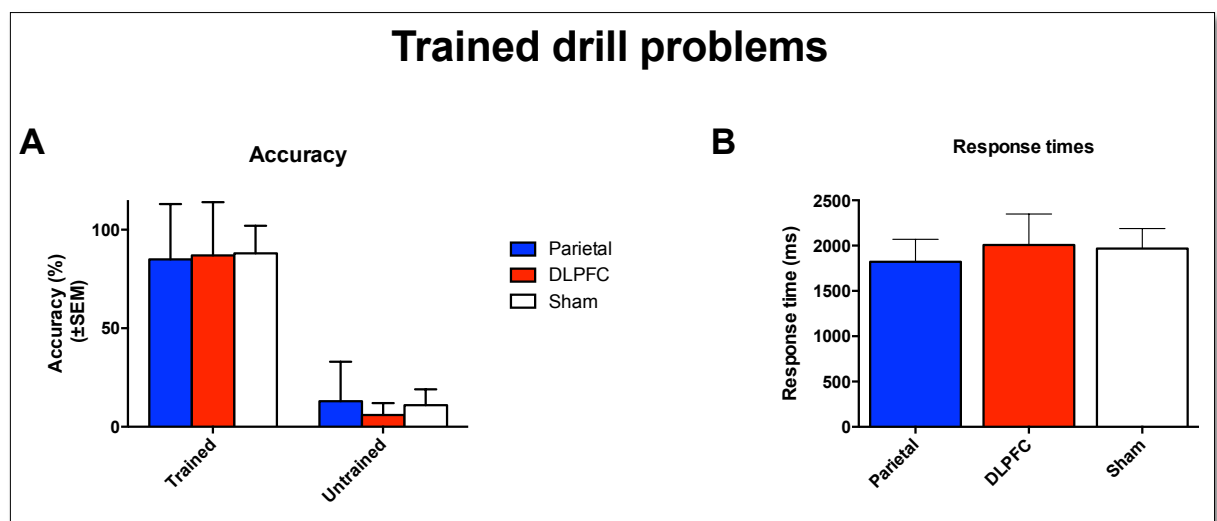


Figure 2.4

Drill testing. There was no effect of stimulation on trained problems for A) accuracy ($p=.45$) or B) response times ($p>.26$).

2.4 Discussion

In this study, I used a within-subject design to reduce inter-individual variation in cognitive abilities (Snowball et al., 2013). This reduced a number of factors that can affect the response to tES (Krause & Cohen Kadosh, 2014). Since

the within-subject design did not allow for the control in initial task performance, I instead controlled for basic mathematical abilities using a standardized test battery. In line with previous results, I found that DLPFC stimulation significantly improved arithmetic learning transfer, but in addition, I found that parietal stimulation showed significantly higher accuracy during training compared to sham and a trend towards higher accuracy compared to DLPFC stimulation. This was despite slow response times on the first training block on day 1 for both tRNS conditions, which was unexpected. The transfer effect was specific to DLPFC stimulation, whereas the training effect was specific to parietal tRNS.

Despite the small sample size and the inability to directly compare the results with previous findings, I found beneficial effects for parietal tRNS on arithmetic performance, which were not found previously (Snowball et al., 2013). This is especially interesting given that accuracy was now improved, which is not the most common result in tES research (Krause & Cohen Kadosh, 2013; Pascual-Leone, Horvath, & Robertson, 2012). In most cases, response times are the only improved measures. Since I hypothesized earlier that individual levels of cortical excitation determines the response to tES (Krause & Cohen Kadosh, 2014; Krause, et al., 2013), I investigated the link between mathematical abilities and non-invasive measures of cortical excitation (glutamate and gamma-aminobutyric acid, GABA) directly in brain areas related to mathematical abilities (frontal and parietal cortices) in both the developing and the adult brain (see 'Inhibition, excitation, and cognitive achievement in the developing brain'; and 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation').

2.5 Limitations

In a systematic review about tES effects on working memory (specifically n-back tasks), it was discovered that between-subject studies are more successful in finding stimulation effects, but not within-subject cross-over designs (Brunoni & Vanderhasselt, 2014). For example, on the tower of London task (TOL), which involves planning steps ahead, cathodal tDCS to the left DLPFC improved performance at acquisition and early consolidation

stages, and anodal tDCS improved later stage performance in a within-subject study design (Dockery, et al., 2009). This study demonstrated a clear effect of the stimulation order. It is possible that some tasks might be more prone to repeated administration and practice effects, such that the stimulation effect can interact with the order of stimulation conditions. Even though the current study may suffer from a certain degree of practice effects, the order of stimulation conditions was carefully balanced across the sample to avoid systematic repetitions. Furthermore, the wash-out period here involved two weeks, whereas most within-subjects designs have shorter periods in the order of minutes. In addition, the sample size was very small in the current study. I would like to note, however, that the recruitment of tES-safe participants who are able to attend to the particularly strict schedule of the current study was challenging. Therefore, testing larger sample sizes in this case was not possible within the time available.

Another potential problem in the current participant sample is the close to ceiling effect on calculation problems that were extensively practiced for five days, such that a valid interpretation of the tRNS on accuracy measures is difficult. As discussed previously (supplemental materials in Snowball et al., 2013), participants have likely prioritized accurate performance over response speed, and this has been shown to yield an effect in the non-prioritized performance variable (Pachella, 1974). This is reflected in their high accuracy rate despite the fluctuations in response times across the five days, which occurred due the reduction of the response time window. These effects are relatively difficult to avoid in above-average calculators. As expected, performance on trained transfer problems was better than on untrained problems, such that this difference also needs to be taken into consideration when participants are selected for participation in complex learning studies.

The next step would be to train participants who start of less proficiently. It is likely that training effects will be stronger in that case, due to the avoidance of ceiling effects. For example, it is possible that tRNS can indeed induce transfer effects when administered to the parietal cortices, but in this case, this effect may have been masked by the ceiling effect. It is also notable that the terms 'calculation' and 'drill' do not refer to 'normal' or real-life learning strategies. Even highly skilled individuals use different strategies to solve the

same arithmetic problems (Dowker, 1992), which affects the ecological validity of studies on many high-level arithmetic abilities.

2.6 Conclusions

I partly replicated previous results showing beneficial effects of tRNS to the DLPFC for arithmetic learning (Snowball et al., 2013). This emphasizes the potential of tRNS to reliably improve cognitive abilities in different individuals under different circumstances when applied to the right brain area during arithmetic training. The fact that I found additional effects using parietal tRNS demonstrates that results and interpretation can be much improved when reducing the influence of individual factors, such as baseline differences and strategy use, on the outcome variables, albeit the robustness of the parietal tRNS results should be examined in a replication study. Individual factors are often neglected in the field but are crucial in order to establish successful neuro-intervention and cognitive training designs. It is likely that the consideration of such baseline differences are not specific to cognitive training but may also be beneficial for functional domains, such as basic motor performance and clinical research involving patterns of patient symptoms.

3 Inhibition, excitation, and cognitive achievement in the developing brain

Abstract

Academic achievement throughout child development is a key predictor of academic achievement later in life. High numbers of children struggling with numerical learning require a better understanding of the underlying mechanism of such developmental cognitive difficulties. Here I examined the link between cognitive abilities and neurochemicals associated with learning and experience-dependent brain plasticity. I assessed performance on standardized mathematics and reading tests in children and adults, and correlated them with *in vivo* noninvasive measures of glutamate/ GABA (gamma-aminobutyric acid), as an indicator for cortical excitation/inhibition (E/I). Using single-voxel magnetic resonance spectroscopy (MRS), I quantified GABA and glutamate in prefrontal and parietal regions. These regions have been strongly associated with numerical cognition, and are known to undergo a trajectory with anterior-to-posterior recruitment during development. Glutamate/GABA in the prefrontal cortex was lower in the frontal cortex compared to parietal and occipital areas. Furthermore, I revealed a dissociation in the relationship between IFG glutamate/GABA ratios and cognitive abilities of children and adults: mathematical achievement scores based on a standardized test and non-standardized number-space mapping abilities were linked to glutamate/GABA ratios only in the child prefrontal cortex. Superior mathematical abilities were related to higher glutamate/GABA ratios in children, which was not driven by glutamate or GABA alone. Further analyses indicated that these effects were specific to mathematics, as verified by a standardized test for reading. These relationships were not found in adults and the correlations differed significantly between groups. My findings link high-level cognitive functions to neurochemicals in a mathematical brain network in the developing, but not the adult brain. This neurochemical

approach therefore only partly reflects findings from other forms of MRI research. My research approach offers the possibility to further investigate the link between neurochemical balances and cognition across development, in order to aid early diagnosis and develop targeted neurointervention. The aim is to better understand mechanisms of efficient cognitive development and potentially to improve future educational and occupational prospects of individuals with otherwise low chances.

3.1 Introduction

Academic achievement – for example in the domains of literacy and numeracy – is a key predictor of success in school, higher education, later careers, and social mobility (Beddington et al., 2008; Ritchie & Bates, 2013). Moreover, the negative effects of poor attainment in these critical domains are reflected not only in individual life outcomes, but also in the welfare of society as a whole. They are therefore of public concern (Butterworth & Kovas, 2013; Gross, et al., 2009). Previous studies have linked cognitive and academic achievement indices to brain development (Butterworth & Kovas, 2013). However, the current understanding of the developing brain and cognition is mainly restricted to functional brain activity and structure (Butterworth & Kovas, 2013; Kaufmann, et al., 2011; Mills & Tamnes, 2014). Better understanding of brain and cognitive development can be aided by the use of different brain imaging methods that allow investigation of how different aspects of neural functioning contribute to cognition (Cohen Kadosh, 2011). Observations at the structural level, measured by magnetic resonance imaging (MRI) techniques are often difficult to interpret in a mechanistic way and the underlying mechanisms of neuroplasticity can be better understood at a deeper biological level (Zatorre, et al., 2012). Two neurochemicals that play a crucial role in neuroplasticity are glutamate and gamma-aminobutyric acid (GABA). These are the brain's major excitatory and inhibitory neurotransmitters, respectively (McCormick, 1989), and are critically involved in learning, memory, and neuroplasticity (Collingridge & Bliss, 1987; Nishiyama, Togashi, Aihara, & Hong, 2010). The interaction between glutamate and GABA, as opposed to both in isolation, is important to consider for the following reasons: the interaction between inhibitory and excitatory synaptic inputs of a cortical neuron eventually determines the neuron's pattern of response, e.g. by refining its tuning towards certain stimuli, or by becoming selective to new features of the stimuli (Oswald, Schiff, & Reyes, 2006; Zhang, Zhou, & Tao, 2011). The resulting neural response to a stimulus in question is therefore particularly important for successful learning and cognitive outcomes. Furthermore, animal models have suggested that across development, brain excitation/inhibition (E/I) balances change due to

refinements in the strength of either the intracortical inhibition (Dorrn, Yuan, Barker, Schreiner, & Froemke, 2010) or excitatory (Sun et al., 2010) neuronal inputs, such that the relative balance shifts across development. As suggested by these animal studies, imbalances in E/I may not only impair animal behavior but are also hypothesized to affect human cognition (Yizhar et al., 2011). The possibility of linking neurochemical markers to cognitive development, aside from its impact on basic research, would provide future opportunities to apply early neuro-cognitive interventions before problems in cognitive development can become hardwired. In order to make a first step towards such approach, I therefore investigated whether glutamate/GABA ratios measured with non-invasive MRI-based were linked with cognitive abilities in typically developing children and adults.

Recent advances in magnetic resonance spectroscopy (see 'MRS; Introduction; Magnetic resonance spectroscopy') allow the reliable quantification of both glutamate and GABA in a brain area of interest, which has until recently been technically challenging (Mekle et al., 2009). This allowed me to examine the relationship between cortical E/I (glutamate/GABA) in children and adults *in vivo* (example spectrum, see Figure 3.2). While neurotransmitter activity itself cannot be assessed noninvasively in humans, MRS provides a unique possibility to investigate the role of glutamate and GABA in brain areas known to be involved in the cognitive function in question. Such approach may also provide a valuable measure of typical and atypical neurocognitive functioning (Silveri et al., 2013). Since both glutamate and GABA are associated with the intensity of the BOLD response (blood oxygenated level dependent), as well as functional connectivity between brain areas and cognitive functioning (Enzi et al., 2012; Hu, Chen, Gu, & Yang, 2013; Northoff et al., 2007; Stagg et al., 2014), I expected that both neurochemicals at rest would be related with mathematical abilities in brain areas known to be involved in numbers and mathematics. I assessed standardized mathematical achievement and lower-level spatial-numerical skills using an established computerized number-space mapping paradigm, in children (7-10 years) and adults (20-23 years). To examine whether potential associations between cortical E/I and cognition were

domain-specific (i.e., mathematics) or domain-general (i.e., general levels of cognitive functioning), I also assessed standardized reading abilities.

Previous functional neuroimaging studies revealed that children recruit frontal brain areas more heavily during mathematical processing, whereas adults show more pronounced activation in parietal areas during the same type of calculation tasks (Kaufmann, et al., 2011; Rivera, Reiss, Eckert, & Menon, 2005). I therefore hypothesized that E/I in frontal and parietal areas would be associated with individual cognitive achievement depending on the developmental stage. In particular, based on these findings, I predicted that variations in E/I within the age-specific region (child inferior frontal gyrus (IFG) and adult intraparietal sulcus (IPS)) would be associated with mathematical, but not reading achievement, as the latter is known to rely on different brain regions (Costanzo, Menghini, Caltagirone, Oliveri, & Vicari, 2013).

3.2 Methods

3.2.1 Participants

Fourteen children (age range 7-10 years, age: mean=8.9 years, SD=.74) and 15 adults (age range 20-23 years, age: mean=21 years, SD=1) participated in the study. An additional two children were tested, but excluded from further analysis due to extreme movement artifacts (one child) and technical issues (one child). Four adults showed strong lipid contamination in the control region (IOG) and were therefore excluded from the correlational analyses. All participants were right-handed females and reported no history of neurological or psychiatric diseases. Participants were compensated for their time and effort with cash for the adults or a book or iTunes voucher for the children and travel expenses for their primary caregiver. Prior to the testing, informed consent was taken from the adult participants and from the primary caregiver in the case of the children. Written assent was also obtained from the children themselves. The study was approved by the Central University Research Ethics Committee at the University of Oxford.

3.2.2 Mathematical assessment

I assessed basic mathematical abilities in all participants (see ‘Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation; Standardized mathematical assessment’).

3.2.3 Number-space mapping task

A computerized version of the task assessing spatial numerical processing was used (Cohen Kadosh, Soskic, et al., 2010; Thompson, Nuerk, Moeller, & Cohen Kadosh, 2013). Performance on this task is closely related to mathematical achievement (Booth & Siegler, 2006) and was therefore selected to confirm my results from my standardized test (WIAT[®]-II^{UK}). The number-space mapping task was presented on a 17-inch Dell computer screen on a black background and participants were seated at a distance of 60cm. A horizontal line from the left to the right side of the screen represented a continuous number line from zero to one thousand. The target number, which was to be placed on the number line, was presented in both left and right upper corners of the screen to avoid spatial biases. Participants were instructed to indicate on the line by mouse click where they thought the target number belonged. Twenty-two trials were preceded by 7 practice trials in which feedback in the form of a red circle representing the correct location on the number line was provided. No feedback was given during the test trials. The target numbers used were 3, 7, 19, 52, 103, 158, 240, 297, 346, 391, 438, 475, 502, 586, 613, 690, 721, 760, 835, 874, 907, and 962.

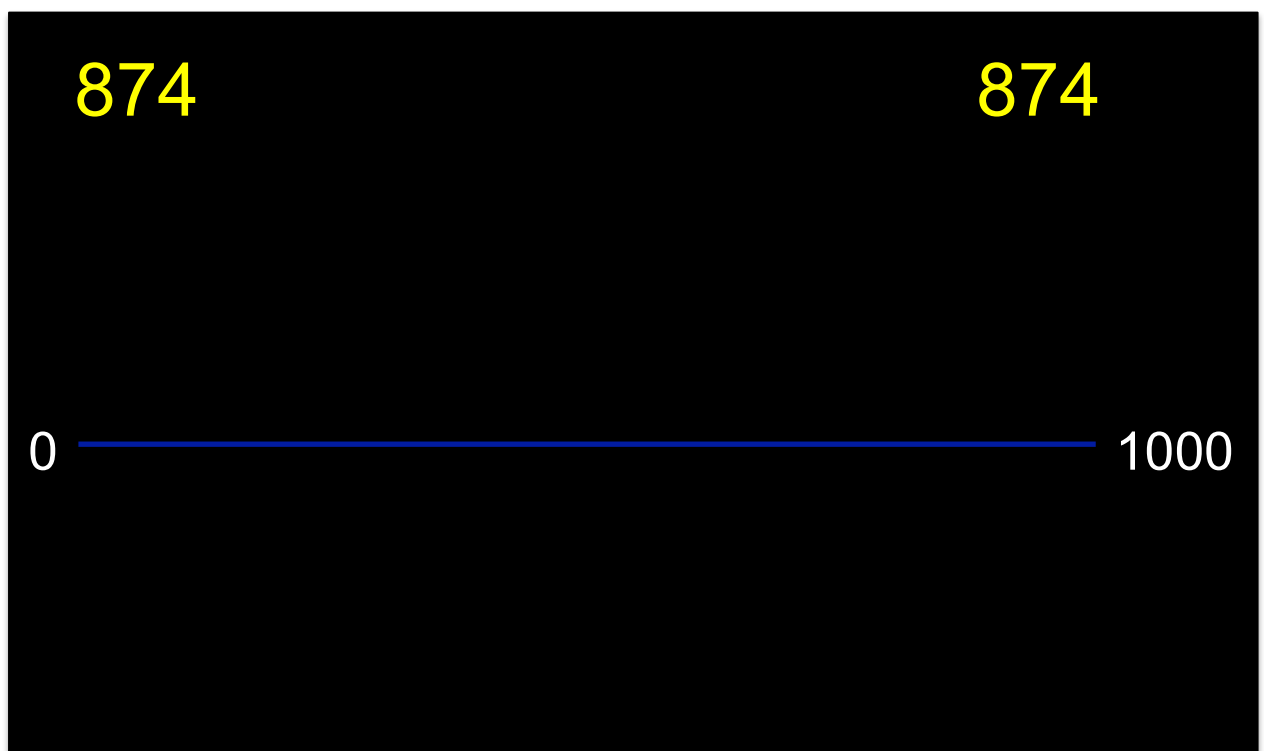


Figure 3.1

Number-space mapping task. The mouse cursor had to be placed where the participant thought 874 is on a line ranging from zero to one thousand. The smaller the difference between the correct and the indicated position, the higher the accuracy.

3.2.4 Reading Skills

Word reading performance was assessed using the Sight Word Efficiency (SWE) and Phonemic Decoding Efficiency (PDE) subtests of the Test of Word Reading Efficiency (TOWRE) (Torgesen, Wagner, & Rashotte, 1999). Sight Word Efficiency (SWE) assesses the reading and pronunciation of written English words, whereas Phonemic Decoding Efficiency (PDE) measures the correct pronunciation of non-words according to English pronunciation rules, such as 'plinders' and 'thundelp'. The number of words read within 45 seconds comprised the raw score, which was then converted into a standard score. Both subtests were combined into a composite standard score, which was used as a measure of word reading efficiency in the current study.

3.2.5 MRS

Single-voxel MRS data were acquired using a 3T Verio system (Siemens Healthcare, Erlangen, Germany) at the Oxford Centre for Functional MRI of the Brain (FMRIB). A 32-channel head coil (Siemens Healthcare, Erlangen, Germany) was used. T1-weighted MR images with a slice thickness of 1mm were acquired (MPRAGE; magnetization prepared rapid gradient echo) with TR=2040ms, TE=4.68ms, TI=900ms (inversion time), and a flip angle of 8 degrees. The localized MRS sequence SPECIAL (spin-echo full-intensity acquired localized) was used to acquire 128 averages with a TR=4000ms, a TE=8.5ms, a bandwidth of 2000Hz, and 4096 points (Mekle, et al., 2009; Mlynarik, Gambarota, Frenkel, & Gruetter, 2006). Water suppression was performed using VAPOR (variable power radio frequency pulses with optimized relaxation delays) (Tkac & Gruetter, 2005). Using this sequence, I was able to separate glutamate from glutamine and GABA from macromolecules (Mekle, et al., 2009) (see example spectrum Figure 3.2). Three 2x2x2cm voxels of interest were manually localized on axial and coronal slices and placed over the right intraparietal sulcus (IPS) and the left inferior frontal gyrus (IFG). Single-voxel MRS requires the a priori selection of the volume of interest based on the individual's brain anatomy, which I localized manually while the participant was in the scanner (voxel location, see Figure 3.3). Due to time constraints, current single-voxel MRS studies usually examine one or two regions at most. The areas were selected a priori based on the available literature on mathematical cognition. Signal contamination from outside the voxels was eliminated by performing outer volume suppression (OVS). Due to the long acquisition time per voxel (~15 minutes including sequence planning and shimming), three voxels was the maximum number of assessments that I considered tolerable for young children, as the sequence added up to one hour total acquisition time.

3.3 Procedure

Each participant first underwent 1 hour of MRI scanning (including the acquisition of a structural scan and the three voxels of interest that were selected a priori), during which participants were able to watch an unrelated

video. All participants were then invited to participate in a separate behavioral testing session (two hours). Tests for mathematical abilities were followed by the reading test. The order of the tasks administered was the same across all participants. Children were compensated for their time and effort with a £20 book voucher and adults with a comparable cash amount.

3.4 Analysis

3.4.1 MRS data

Data were preprocessed as described elsewhere (Near et al., 2013), using automated MATLAB (Natick, MA, USA) routines for the removal of motion-corrupted averages, and the correction of frequency and phase drifts. Linear Combination Model (LCM) (Provencher, 2001) version 6.2-2B with a simulated basis set including a simulated macromolecule signal was used to analyze MRS data. Spectral quality was ascertained by average Cramer-Rao lower bounds (CRLBs) of less than 17% per region and the spectral line widths of each individual spectrum was below 6Hz³. Since MRS is a relative quantitation method, GABA and glutamate were referenced to total creatine (GABA/creatin and glutamate/creatin ratios presented here). Corrections for grey and white matter and cerebrospinal fluid in the voxel were not performed for glutamate/GABA ratios, as the application of the same equation to GABA and glutamate would have cancelled out in the ratio (glutamate/GABA). I used FMRIB's automated segmentation tool (FAST) to segment the structural scans into 3D volumes of grey and white matter (GM and WM, respectively) and cerebrospinal fluid (CSF) (Zhang, et al., 2011). In order to control for differences in tissue proportions in each voxel, GABA and glutamate

³ In order to control for variations in cortical E/I outside the main mathematical brain network, I also selected a third region, namely the right inferior occipital gyrus (rIOG, see Figure 1.4). Due to poor data quality in the IOG, the VOI had to be excluded from the analysis. Out of 14 acquired voxel measurements per age group in this region, 2 for the children and 4 for the adults had to be excluded. Furthermore, the standard error (SEM) in the IOG was generally high (IPS mean=7.83±.38; IFG mean=5.33±.24; IOG mean=11.54±2.25). The quality problem can be explained by the close proximity of the voxel to the cortical surface and ventricles, which is more susceptible to distortions and movement artifacts.

concentrations scaled to total creatine (creatine plus phosphocreatine; Cr+PCr) were corrected by multiplying GABA and glutamate with $[GM]/([GM]+[WM]+[CSF])$, while creatine was multiplied by $([GM]+[WM])/([GM]+[WM]+[CSF])$. Note that MRS is a relative rather than an absolute quantitation method. For the analysis of creatine effects, creatine was scaled to total creatine ($Cr/(Cr+PCr)$).

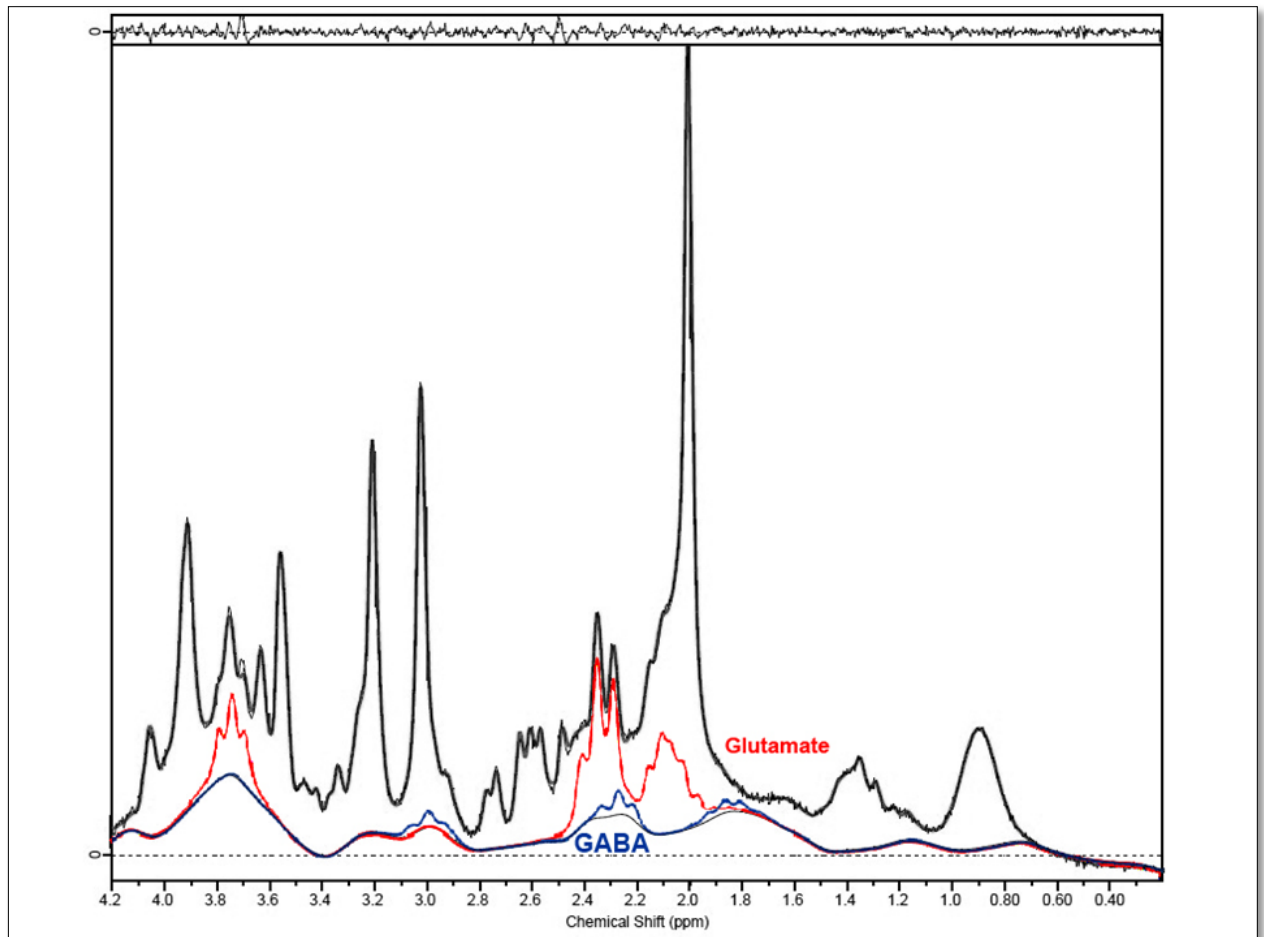


Figure 3.2

An example spectrum of a child IFG voxel. The main area shows the GABA (blue) and glutamate (red) curves in relationship to the whole spectrum of metabolites acquired. The top bar reflects the noise subtracted from the spectrum. The chemical shift is indicated on the x-axis and the area under each curve demonstrates the quantity of the neurotransmitter. Both neurotransmitters have three major peaks: GABA peaks at 3.0, 2.3 and 1.9ppm and glutamate at 3.8, 2.3 and 2.1ppm.

3.5 Statistics

Spearman's correlations (r_p) were computed using SPSS Statistics 20. Outliers more than 2.5 standard deviations from the mean were removed. This corresponded to a maximum of one data point on some of the tasks in the child sample (a total of 2.6%) and 0% in the adult sample⁴. I used Fisher's Z transformations to compare whether correlation coefficients differed between children and adults.

3.6 Power analysis

Using G-Power 3.0, I computed the sample size required for optimal power (95%) in the current statistical analyses. The minimum accepted effect size was set at .25 ($\eta_p^2=.06$) and a correlation between repeated measures assumed to be $r=.5$. For a repeated measures interaction (within-between group), the required total sample size should be $N=54$ for a power of .95. For a power of .8, an adequate sample size would be $N=34$. For a power of .95 to investigate the between-subject factor group, the sample size would even have to be between $N=158$ ($N=98$ for a power of .8). For the correlational analysis, a total sample size of $N=37$ would be required to reach a moderate effect size of $R^2=.3$ (Pearson's $r=.55$).

3.7 Results

I first examined differences in cortical glutamate/GABA as a function of group and brain area using repeated measures analysis of variance with VOI (IPS, IFG) as a within-subject factor and age (child/adult) as a between-subject factor (see Figure 3.3). The main effects of VOI ($F(1,26)=51.02$, $p<.001$, $\eta_p^2=.66$) and age ($F(1,26)=5.42$, $p=.03$, $\eta_p^2=.17$) were significant. Glutamate/GABA ratios were generally higher in the IPS than the IFG, and children had higher ratios than adults (Figure 3.3). There was an interaction between group and VOI for GABA ($F(1,26)=4.97$, $p=.04$, $\eta_p^2=.16$) and glutamate ($F(1,26)=11.68$, $p=.002$, $\eta_p^2=.31$) separately. Children had higher GABA levels than adults in the IPS ($F(1,26)=4.59$, $p=.04$, $\eta_p^2=.15$), but there

⁴ One child outperformed the group (2.7 standard deviations above the group mean) on the numerical operations subtest and therefore had to be excluded from the respective correlational analyses.

was no difference in the IFG ($p=.32$). Children also had higher levels of glutamate in the IPS ($F(1,26)=112.88$, $p<.001$, $\eta_p^2=.81$), but also in the IFG ($F(1,26)=33.88$, $p<.001$, $\eta_p^2=.56$) (Figure 3.4).

With respect to the cognitive measures, the groups did not differ in their standardized mathematical achievements scores (all $ts(26)<.96$, all $ps>.34$). However, adults showed significantly superior performance in the non-standardized number-space mapping task ($t(26)=4.44$, $p<.001$, $d=1.74$), replicating previous findings (Siegler & Opfer, 2003). In contrast, children's standard scores for reading were superior to the adults' ($ts(26)>2.95$, $ps<.008$, $ds>1.16$). Critically for the correlation analysis, the variance in the different mathematical tests ($ps>.18$) as well as glutamate/GABA in each region ($ps>.35$) did not differ between the two groups.

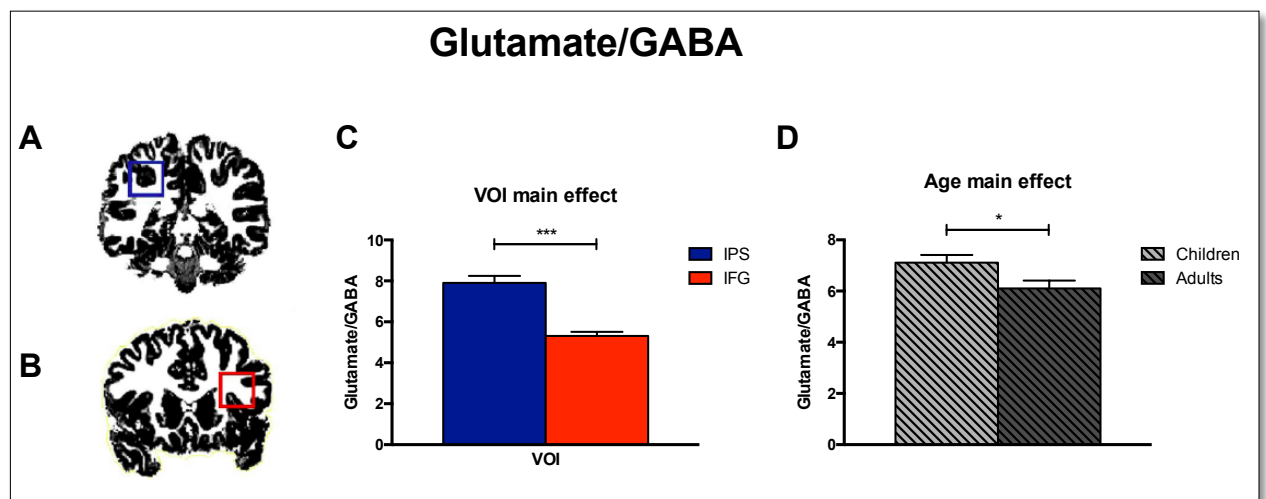


Figure 3.3

Cortical Glutamate/GABA in the child and adult brain. Voxel positions for A) right IPS and B) left IFG. C) Main effect of VOI on Glutamate/GABA: the ratio was higher in the IPS than the IFG. D) Main effect of age on Glutamate/GABA ratios: ratios were higher in children than adults ($*p<.05$, $***p<.001$).

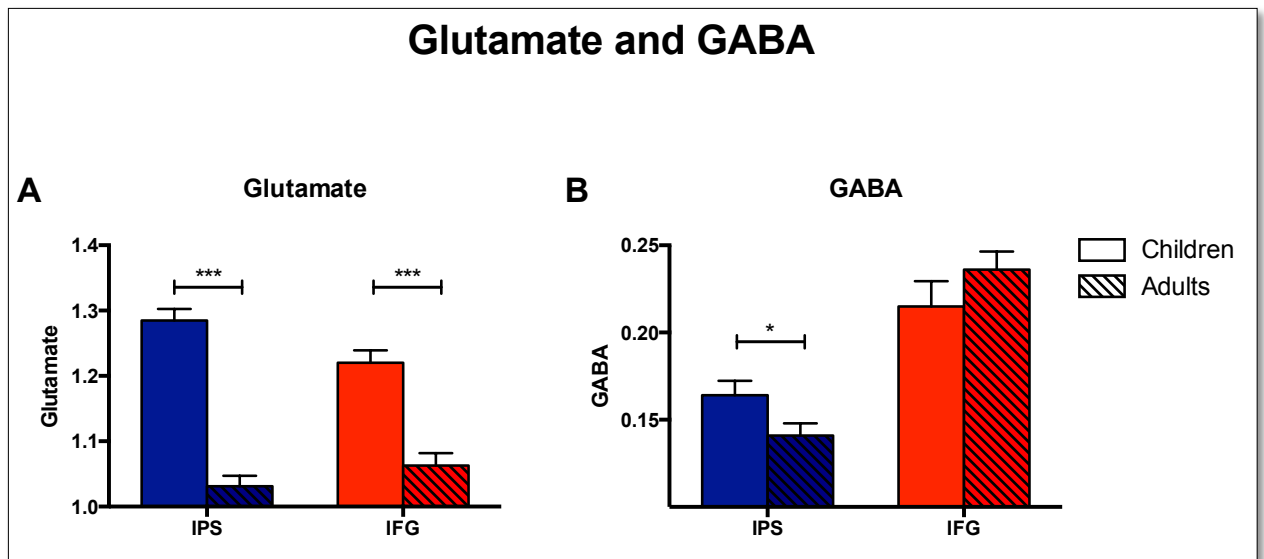


Figure 3.4

Glutamate and GABA concentrations per VOI in children and adults. Children had higher levels of A) glutamate in the IPS and IFG and B) GABA in the IPS, compared to adults. It appears that GABA is more stable across development than glutamate.

3.7.1 Adult IPS

There was no relationship between Mathematics and glutamate/GABA in any region in the adults (see Table 3.1).

3.7.2 Child IFG

The composite score correlated positively with glutamate/GABA ratios, which was similar for the mathematical reasoning subscale, but did not reach significance for numerical operations (Table 3.1). Furthermore, using a non-standardized test, I confirmed the direction of the relationship by finding a trend for number-space accuracy ($r_p = .51$, $p = .06$). Reading was not related to glutamate/GABA in the IFG ($r_p = .28$, $p = .33$). The effects for mathematical abilities were limited to the IFG and were not found in the child IPS. Further analyses indicated that the associations between glutamate/GABA and cognitive abilities could not be explained by concentrations of glutamate or GABA separately (see Table 3.2).

	Numerical operations	Mathematical reasoning	Mathematics composite	Number- space ACC	Reading
Children					
IFG	.5	.6*	.65*	.51 ^{trend}	.28
IPS	.51	-.12	.28	-.02	.33
Adults					
IFG	-.42	-.25	-.36	.22	.2
IPS	-.15	.07	-.02	.11	.5
	<i>Fisher's test (Z) for the equality of correlation coefficients children vs. adults</i>				
IFG	2.28*	2.22*	2.64**	0.8	0.2
IPS	1.63	-0.45	0.7	-.31	-0.48

Table 3.1

Non-parametric Spearman's correlation coefficients between glutamate/GABA ratios and mathematical and reading abilities. In the child IFG, superior mathematical abilities were associated with higher ratios of glutamate/GABA. This effect was specific to the IFG and was not found in the child IPS or in adults. The bottom panel shows the results from a Fisher's transformation: the correlation coefficients between child and adult IFG correlations were significantly different for the standardized mathematical batteries only (* $p < .05$; ** $p < .01$).

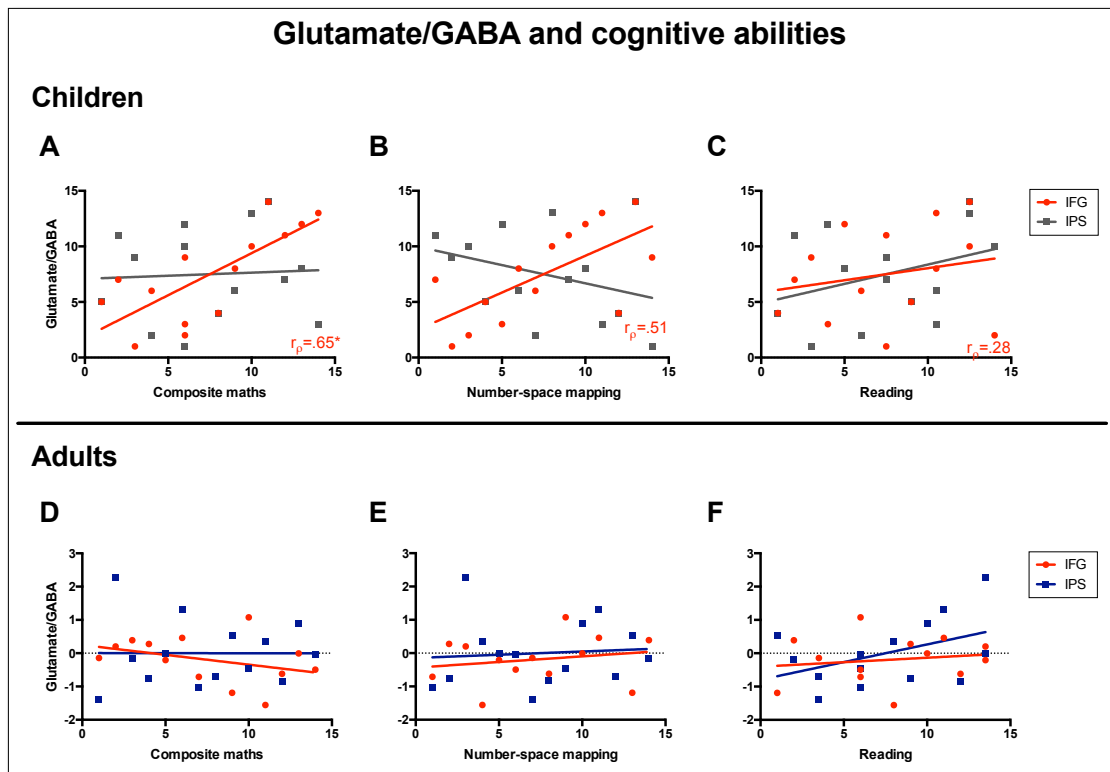


Figure 3.5

Spearman's correlations between ratios of glutamate/GABA and cognitive abilities. A) In the IFG of children, standardized mathematical abilities were positively associated with glutamate/GABA and there was a trend for B) unstandardized mathematical abilities, but not for C) reading. There were no correlations in the IPS. In the adults, none of the cognitive measures correlated with glutamate/GABA in the IFG or IPS (D-F). (* $p < .05$).

	Numerical operations	Mathematical reasoning	Mathematics composite	Number-space ACC	Reading
Children					
IFG					
Glutamate	-.31	.1	-.05	.22	-.16
GABA	-.31	-.22	-.29	-.08	-.41
Adults					
IPS					

Glutamate	.33	.26	.29	-.02	.19
GABA	.25	-.006	.1	-.22	-.16

Table 3.2

Metabolite concentrations in the child IFG and adult IPS. The association between mathematical abilities and glutamate/GABA in the child IFG was not driven by GABA or glutamate alone. For adults, none of the metabolites in the IPS was associated with mathematical abilities.

3.8 Discussion

Using MRS, I quantified glutamate and GABA noninvasively in children and adults. I revealed a dissociation between groups for the relationships between glutamate/GABA and cognitive achievement. E/I was associated with cognitive abilities in the left frontal cortex of children, a region known to be heavily involved in the cognitive tasks in question at the age of the sample. Superior mathematical abilities were associated with higher E/I (i.e. enhanced cortical excitation relative to inhibition). This was consistent across different measures for mathematical abilities and could not be explained by excitation (glutamate) or inhibition (GABA) alone, which suggests that the interplay between excitation and inhibition may provide a more sensitive measure for cognitive performance. There was no such relationship in this region with reading abilities, and no such relationships could be found in the adult mathematics network. Independent of the area, children showed higher glutamate/GABA ratios than adults. When investigating the concentrations of glutamate and GABA separately, it became apparent that the age differences per brain area were more pronounced for glutamate. Glutamate was higher in both areas in children, but GABA was only higher in children in the IPS but not the IFG. It is therefore possible that the lack of an association between mathematics and glutamate/GABA in the adult IFG is due to decreasing levels of glutamate across development. However, a larger scale longitudinal study would provide more insight to answer this question.

Maturation processes in the cortex have been associated with reduction in grey matter from childhood towards adulthood, starting with posterior, and

progressing toward frontal brain areas (Gogtay et al., 2004). These processes demonstrate that frontal areas, where higher-level cognitive abilities develop, mature last. At the neurochemical level, higher cortical excitability is associated with higher degrees of neuroplasticity, which then decreases over the course of brain development from childhood to adulthood (Mills, Lalonde, Clasen, Giedd, & Blakemore, 2012; Shaw et al., 2006). Such enhanced excitability enhances the capacity for learning and allows for greater flexibility of the structure of cortical connections (Knudsen, 2004). This period of enhanced learning capacity during development may be regulated by E/I balances and may determine cognitive processing efficiency (Hensch & Bilimoria, 2012). Atypical E/I is thereby associated more profound and distinctive cognitive deficits, which has been shown in animal models of neuropsychiatric disorders (Rubenstein & Merzenich, 2003; Yizhar, et al., 2011).

The left inferior frontal gyrus (IFG) and the right intraparietal sulcus (rIPS) are strongly involved in numerical processing, and are differentially recruited by children and adults during arithmetic tasks. A shift from more frontal to more parietal processing could be found in numerous functional neuroimaging studies during the transition from childhood to adulthood (Kaufmann, et al., 2011; Rivera, et al., 2005). Even though I was able to show that glutamate/GABA ratios were related to standardized and non-standardized mathematics measures in children, I found no evidence for the involvement of the adult parietal cortex. There are several possible explanations for this dissociation in results: 1) I was only able to measure neurochemicals in one voxel in the parietal cortex (the right IPS), but it is possible that the relationship differs in the left hemisphere or in other parietal areas. 2) It is also possible that glutamate/GABA plays a role in mathematical abilities during development and learning (periods of enhanced plasticity), but plays less of a role in adulthood, when abilities and plasticity are more stable (periods of reduced plastic capacity). 3) Larger samples and wider age ranges are required to gain better insight into the developmental trajectory of such neurochemical-behavior relationships.

Despite the poor spatial resolution of MRS compared to functional or structural neuroimaging methods, my results demonstrate the usefulness of

MRS to study neurochemicals in the field of neurocognitive development. This research approach can be used to investigate neuro-cognitive development at a deeper biological level than currently possible. MRS methodology is advancing and will lead to improved spatial resolution in the future (Di Costanzo et al., 2007; Near, et al., 2013; Passeri, Mazzuca, & Del Bene, 2014). I showed here that MRS measures of glutamate/GABA need not only be used to distinguish different stages of development at the group level (children from adults), but also to investigate within-group individual variations in cognition. Future studies should aim to investigate whether glutamate/GABA can serve as a useful marker for atypical cognitive development. Learning studies have demonstrated that glutamate and GABA can be modulated, and noninvasive brain stimulation can enhance such effects (Floyer-Lea, et al., 2006; Kim, Stephenson, Morris, & Jackson, 2014; Sampaio-Baptista, et al., 2015; Stagg et al., 2009). While this work is currently limited to motor performance, in the chapter 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation', I investigated whether the improvements in mathematical abilities under non-invasive brain stimulation also lead to changes in glutamate and GABA in the brain's mathematics network. Such techniques would be useful to firstly, understand the relationship between brain mechanisms and cognitive learning, and secondly, to work towards neuro-interventions to remove the brakes on learning-related plasticity in developmental disorders. Especially at earlier stages of development and during enhanced plasticity, such an intervention would be most efficacious (Cramer et al., 2011). For example, at the academic level, ideal interventions should be made before a child lags behind in her educational requirements (Krause & Cohen Kadosh, 2013). Therefore, cortical properties associated with an individual's current capacity are crucial to consider to custom-design such targeted interventions (Krause & Cohen Kadosh, 2014).

In summary, the ability to expand the link between brain and cognition beyond the functional and structural levels to neurochemistry is important as it could enable the development of neurointervention methods that can modulate neurochemicals with the aim to improve learning and neuroplasticity. It could

promote the development of interventions targeted at different age groups and thereby improve the lives of those with atypical brain development.

4 The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance

Abstract

Alterations in excitatory and inhibitory neurotransmitters (glutamate and GABA, respectively) have been found in various neurological disorders. However, the neurochemistry of the opposite, namely individuals with prodigious abilities, has not been investigated. I tested the nine-fold world champion in mental calculation, G.M., and compared his neurochemistry to four healthy expert control calculators, who were not prodigies. Here I demonstrate a substantial reduction in frontal glutamate/GABA ratios, which was not found for glutamate or GABA separately, measured noninvasively with magnetic resonance spectroscopy. I suggest that regional noninvasive measures of glutamate/GABA ratios can identify extreme cases of cognitive skill levels.

4.1 Introduction

The early examination of mental calculators started in the late 1900's, when phrenological and psychological accounts were combined with the neurological observations of a seven year-old boy with exceptional auditory (but not visual) memory for numbers. He could memorize up to 36 digits in a row by hearing his own inner voice utter the digits and being able to recall them even hours later. The boy, called Inaudi, also showed right frontal and left parietal skull protrusions (malformations of the skull bone), which were hypothesized to be due to cerebral hypertrophy, but his brain itself was never investigated (see Nicolas, Guida, & Levine, 2014).

Scientists have long been fascinated with the phenomenon of prodigious abilities. While developmental disorders or acquired brain damage are clearly associated with a lack or loss of abilities, we find extraordinary abilities in prodigies that are far beyond a normal individual's capacity and in most cases cannot be acquired by learning. A lightning calculator is typically an individual who, whenever presented with complex arithmetic problems (often multiplication, root calculation or prime factorization), provides the answer within only a few seconds. A related, but less extraordinary skill is calendar calculation. Asking the individual "What day of the week was September 25th in 1837?", the answer will be "Monday", and is provided within a couple of seconds. This ability, however, can be acquired by practice to a certain extent. Furthermore, quantity estimation is more rarely reported in such individuals but has been observed by the popular neurologist Oliver Sacks in a couple of autistic twin boys. The boys showed an extraordinary perceptual ability to 'see' and not to guess or estimate (i.e. their complete accuracy suggested that they knew the number) large numbers of objects instantaneously. For example, when a match box fell on the floor and the matches were scattered around, the boys immediately exclaimed "111, 37, 37, 37," for the number of matches and its three equal parts it was made up of (Sacks, 1985). Sacks reported that even at age 26, these twins were mentally challenged with an IQ of 60, did not even understand the simplest concepts of calculation (e.g. addition or multiplication), but engaged in hour-long sessions of identifying prime numbers of up to 20 digits long, with unknown or

unobvious strategies. All they could report was that they 'saw' the numbers, suggesting they relied heavily on visual imagination. In addition, they were accurate in predicting the day of the week for the past and coming 40,000 years and Easter for the next 80,000 years. Even though simple calculation strategies can be applied here, it is uncertain how these twins could have gained the solutions without being able to multiply or divide.

Many of the known cases of prodigious abilities stem from individuals with autism, who have one relatively isolated exceptional skill but are otherwise cognitively severely impaired. This phenomenon was originally labeled 'idiot savant', which has been changed to 'savant syndrome' due to its more respectful and accurate term. Explanations range from obsessive practice to the savant abilities, like specific deficits, exemplifying the relative functional independence of different abilities (see Heavey, 2003 for a review). One possible explanation is that these individuals lack higher-level cognitive abilities, which allows them to be highly efficient in very simple and lower-level cognitive abilities (Snyder, 2009). The theory has been supported by interrupting brain functioning in certain brain regions of normal participants and thereby improving performance on simple cognitive or perceptual tasks (Snyder, Bahramali, Hawker, & Mitchell, 2006; Snyder et al., 2003). However, it is difficult to give a conclusive explanation for savant abilities without more knowledge of the existence, extent and nature of similar prodigious abilities in individuals who do not have impaired cognitive abilities. In the current study, neurochemical data from a high-functioning expert calculator with prodigious abilities will be presented.

Mental calculation is a highly complex concept involving a variety of brain functions, including a good understanding of numbers and quantities, memory retrieval of arithmetic facts (ranging from multiplication tables to knowing that $\pi \approx 3.14$, as opposed to calculating it), the manipulation of numbers in which intermediate calculation steps need to be held in memory and updated with concurrent results, and the comprehension and verbalization of number words. The brain network associated with such complex cognitive abilities is spread widely across the cortex, involving a variety of especially frontal and parietal regions (Arsalidou & Taylor, 2011). Working memory capacity is strongly associated with calculation abilities (Berg, 2008; DeStefano &

LeFevre, 2004; Furst & Hitch, 2000) and can be linked to both the structure and activity in the dorsolateral prefrontal cortex (DLPFC), which is part of the middle frontal gyrus (MFG). For instance, in normal participants, brain activity and gray matter volume in the right middle frontal gyrus can be modulated with working memory training (Olesen, Westerberg, & Klingberg, 2004; Takeuchi et al., 2011).

Unfortunately, from the early cases of prodigious abilities, we have no evidence of the individuals' brain anatomy or functioning (Nicolas, et al., 2014; Sacks, 1985). Nowadays, we compare the brains of prodigies with normal calculators and can more or less accurately report how their brain structure and activity differs from others. The right prefrontal cortex has thereby been identified as one of the key regions in expert calculators (Fehr, Weber, Willmes, & Herrmann, 2010; Pesenti et al., 2001). A different way to investigate the role of certain regions in such extraordinary skills is the use of scientific manipulation techniques. For example, sharp increases in simple perceptual numerical performance (such as in Sacks' twins) have sometimes been found when interfering with ongoing brain activity in the left frontotemporal junction using repetitive transcranial magnetic stimulation (rTMS) (Snyder, et al., 2006). The authors concluded that simple lower-level perceptual abilities could be unmasked, when higher-level functions were turned off. Furthermore, visual memory could be improved by applying excitatory and inhibitory current to the left and right temporal lobes of normal participants, respectively, such that the resulting performance was similar to that observed in autistic individuals (Chi, Fregni, & Snyder, 2010). The authors explained that this effect could be due to a process they call 'paradoxical facilitation', in which the facilitation of one hemisphere and the concurrent inhibition (or decrease in excitation) of the other hemisphere leads to the disinhibition, and thereby facilitation, of the target area in the first hemisphere.

It is important, however, to interpret these results with caution, as most people with damage to left frontotemporal regions do *not* exhibit savant abilities; nor does TMS to these regions always lead to improved calculation performance. Moreover, prodigious calculation abilities have been reported in people without brain damage or known cognitive difficulties (Fehr, et al., 2010;

Pesenti, Seron, Samson, & Duroux, 1999). However, because much research on the cognitive and neural mechanisms associated with prodigious calculation talent has focused on savants with autism or other disorders, it is sometimes difficult to disentangle the characteristics of prodigious calculation as such from the characteristics of concomitant disorders. The present study investigates the characteristics of a high-functioning prodigious calculator. To expand our knowledge of the neural underpinnings of prodigious abilities, I examined neurobiological characteristics that have not typically been studied in this connection: i.e. neurochemistry.

Concentrations of GABA measured by magnetic resonance spectroscopy (MRS) have been found to be altered in neuropsychiatric disorders associated with cognitive impairments, such as schizophrenia, autism and Parkinson's disease (Edden, et al., 2012; Emir, Tuite, & Oz, 2012; Kubas, et al., 2012; Rojas, et al., 2013; Rowland, et al., 2012). MRS is therefore a potential method to identify neuropsychiatric abnormalities in the brain that are otherwise not or not yet observable in structural abnormalities. As such, MRS provides the potential for the use of GABA as a biomarker to identify such disorders based on regional concentrations in the brain. Glutamate and GABA are the brain's major excitatory and inhibitory neurotransmitters and are both strongly implicated in learning and plasticity (Ge & Dani, 2005; Trepel & Racine, 2000). In order for the brain to produce meaningful processing and output, this cortical excitation and inhibition (E/I) must be balanced (Turrigiano & Nelson, 2000). Only an optimal balance between the two opponents allows for a stable network that nevertheless allows for experience-dependent plastic changes (Hess & Donoghue, 1994, 1996). E/I balance for example has been associated with complex cognition, such as decision-making (Jocham, Hunt, Near, & Behrens, 2012).

There is currently comparatively little knowledge of the underlying biological mechanisms associated with the development of prodigious abilities. While previous research has mainly compared healthy to pathological conditions and investigated individual variations in GABA and glutamate, I aimed to investigate differences in glutamate and GABA between a healthy individual with extraordinary cognitive capacity and high-achieving controls with similar interests and backgrounds. Due to the extreme rarity of such individuals, I

compared the current world champion in mental calculation, G.M., with four highly intelligent, age- and education-matched male controls who had similar standardized scores for mathematical abilities but were not prodigies.

Frontal areas, such as the right middle frontal gyrus (MFG) have previously been associated with exceptional calculation skills (Pesenti, et al., 2001). While other areas, such as the intraparietal sulcus (IPS) are also heavily involved in adult calculation abilities, the IPS relying more on quantity processing and the internal spatial representations of numbers may not be too useful for high-level calculation abilities (Kaufmann et al., 2008; Kaufmann, et al., 2011). I therefore investigated GABA and glutamate levels as measures of the capacity for cortical plasticity in the right MFG, the left IPS and in primary visual cortex (V1), as a control region. Since normal, healthy individuals are likely to show differences in brain functioning and structure in a variety of brain areas (Fehr, et al., 2010), I selected four control participants with a high level of mathematical understanding and ability and a history of mathematics in their education.

4.2 Methods

4.2.1 Participants

4.2.1.1 G.M.

G.M. is a male German, 46-year old, high-functioning, healthy calculation expert with no history of neurological or psychiatric conditions. He has been engaging in competitive mental calculation events for more than 25 years and is a member of Mensa. G.M.'s exceptional mental calculation skills have repeatedly been demonstrated in international mental calculation competitions and he holds nine gold medals and several world records in mental calculation. He holds two university doctorate degrees in humanities and is also highly skilled in calendar calculation. I measured his standardized mathematical abilities to be above the 99.8th percentile. He reached the top composite standard score of 143 on the WIAT-II-UK (see 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation; Standardized mathematical assessment').

4.2.1.2 Control participants

Control participants were recruited from the University of Oxford senior staff members in the fields of psychology, mathematics, or physics. All were healthy, male right-handed individuals without a history of neurological or psychiatric disorders, and were highly proficient in mental calculation, as assessed by the WIAT. Participants consented to volunteer in the study and all were scanner safe.

Participant	DB	JA	SF	AB	G.M.
Age	45	49	42	41	46
Years of higher education	11	9	13	6	11
Math composite (std score)	126	128	120	116	143
Math percentile	96	97	91	86	99.8
Study topic	combustion engineering and artificial intelligence	math sciences and physics	psychology, physics, medical physics	physics	math, psychology and education

Table 0.1

G.M. and male control participants matched for age, education and standardized math scores. All participants had a certain involvement in psychology and related sciences.

4.2.2 Standardized mathematical test

To assess basic mathematical abilities, I used the Wechsler Individual Achievement Test (WIAT® -II-UK; Wechsler, 2005). For details, see

‘Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation; Standardized mathematical assessment’.

4.2.3 MRS

Three 2x2x2cm voxels of interest were manually localized on axial and coronal slices and placed over the left intraparietal sulcus (IPS), the right middle frontal gyrus (MFG) and primary visual cortex (V1). The latter served as a control region. Due to the long acquisition time per voxel (~15 minutes including sequence planning and shimming), three voxels that were selected a priori based on the available literature on mathematical cognition. For detailed methods see ‘Inhibition, excitation, and cognitive achievement in the developing brain, MRS’.

4.2.4 Procedure

Written informed consent was received from all participants prior to the beginning of the study. Participants were resting in the MRI scanner and watching a video while a structural scan and three voxels of interest were acquired. Scanning time varied between 60 and 90 minutes. In a single behavioral testing session, basic mathematical abilities were assessed using a standardized test (WIAT-II-UK). Participants were compensated for their time and effort with £10 per hour and travel expenses were covered. The study was approved by the Berkshire Research Ethics Committee.

4.3 Analysis

4.3.1 MRS data

I placed three voxels in the right MFG, the left IPS and V1 (for details see ‘Inhibition, excitation, and cognitive achievement in the developing brain, MRS data’).

4.3.2 Crawford’s modified t-test for single case studies

Since I tested a single case and a small control group, the Crawford and Howell modified t-test was used to compare G.M.’s regional glutamate and

GABA concentrations with those of the four control participants (Crawford & Howell, 1998). In this test, the data (e.g. the glutamate/creatine ratio) from the case (G.M.) is treated as a sample and a modified t-test is applied. The original t-test was reported by Sokal and Rohlf (Sokal & Rohlf, 1995). The degrees of freedom are calculated as:

$$df = N_1 + N_2 - 2$$

In the current study study, the degrees of freedom for one case and four controls accordingly will be $N_{G.M.} + N_{controls} - 2 = 3$. The t-statistic is then calculated by;

$$t = \frac{Glu_{GM} - \overline{Glu}_{controls}}{Standard\ deviation_{controls} \sqrt{\frac{4 + 1}{4}}}$$

Here, the mean of the control group is subtracted from G.M.'s glutamate concentration, and divided by the standard deviation of the control group multiplied by the root of the whole sample size divided by the control sample size. For the MFG, G.M.'s glutamate concentration was =0.93 and the mean sample concentration was =.89, with a standard deviation of 0.09.

$$t = \frac{0.93 - 0.89}{0.09 \sqrt{\frac{4 + 1}{4}}}$$

$$t = \frac{0.04}{0.09 \sqrt{1.25}}$$

$$t = \frac{0.04}{0.1}$$

$$t = 0.40$$

With $df=3$ and a t -statistic=0.40, a two-tailed test is non-significant (the critical value for an alpha of .05 would be at $t=2.78$) (Field, 2009). The software for the modified t -test is freely available and can be found online at:

<http://homepages.abdn.ac.uk/j.crawford/pages/dept/SingleCaseMethodsComputerPrograms.HTM>.

4.4 Results

Regional concentrations of GABA and glutamate concentrations raw and in relation to creatine shown in Table 0.1. Cramer-Rao lower bounds (CRLBs) below 20% indicate the reliability of the acquired concentrations per region. In the MFG and V1, none of the participants had a CRLB of higher than 20% for GABA, indicating good reliability. However, in the IPS, the reliability was less consistent (see Table 0.3). Therefore, IPS data were not further analyzed. Line widths (full-width-half-maximum, FWHM) shown in ppm (parts per million) and in brackets signal-to-noise ratio (SNR). In V1, the SNR was significantly lower in G.M. compared to controls ($p=.02$). MFG glutamate/GABA was significantly lower in G.M. than in the controls ($p=.04$) (Figure 0.1 C, Table 0.2), but not GABA ($ps>.09$) or glutamate separately ($ps>.08$). In order to control for the potential confounding factor of the reference metabolite creatine, I performed the modified t -test on the raw, as well as referenced metabolites. Furthermore, in the MFG, creatine was not significantly different between G.M. and controls ($t(3)=.32$, $p=.77$). Additionally, there was no significant difference between CRLBs ($t(3)=-1.18$, $p=.32$).

No linear association could be found between standardized mathematical abilities and glutamate/GABA in the MFG ($p=.13$) (Figure 0.1 D). Table 0.4 shows Pearson's correlation coefficients between mathematics scores and neurochemical concentrations in an overview. Non-parametric Spearman's correlations revealed that all coefficients were $ps>.26$ for the MFG and $ps>.28$ for V1. It remains to be considered that the sample size is very small. A visualization of the relationship between mathematics and metabolites is shown in Figure 0.1.

Participant	DB	JA	SF	AB	G.M.	<i>t</i> - statistic, <i>p</i> -value
MFG						
GABA						
Raw concentration (CRLB %)	1.13 (17)	1.24 (20)	0.99 (19)	1.45 (14)	1.78 (14)	T=2.66, <i>p</i> =.08
GABA/Cr	0.158	0.148	0.138	.186	0.214	T=2.44, <i>p</i> =.09
GM/WM corrected	.044	.032	.025	.028	.041	T=.94, <i>p</i> =.42
Glutamate						
Raw concentration (CRLB %)	5.93 (3)	7.25 (3)	5.93 (3)	7.95 (3)	7.77 (4)	T=.89, <i>p</i> =.44
Glutamate/Cr	0.829	0.87	0.826	1.021	0.934	T=.46, <i>p</i> =.68
GM/WM corrected	.23	.188	.15	.156	.178	T=-.07, <i>p</i> =.95
Glu/GABA	5.25	5.86	5.98	5.49	4.36	T=- 3.42, <i>p</i>=.04
Linewidth (SNR)	.036 (77)	.044 (67)	.032 (80)	.032 (84)	.024 (69)	<i>p</i> =.15 (<i>p</i> =.4)
V1						

GABA						
Raw concentration (CRLB %)	2.2 (10)	1.75 (11)	1.23 (16)	1.84 (10)	1.59 (10)	T=-.37, p=.74
GABA/Cr	0.279	0.284	0.192	.267	0.268	T=.26, p=.81
GM/WM corrected	.168	.13	.104	.165	.151	T=.27, p=.8
Glutamate						
Raw concentration (CRLB %)	6.97 (3)	5.71 (3)	5.16 (4)	7.39 (3)	5.65 (3)	T=-.56, p=.61
Glutamate/Cr	0.887	0.928	0.805	1.071	0.953	T=.24, p=.82
GM/WM corrected	.533	.426	.437	.661	.539	T=.2, p=.85
Glu/GABA	3.18	3.27	4.19	4.01	3.56	T=-.18, p=.87
Linewidth (SNR)	.036(88)	.028(87)	.028(87)	.032(85)	.032(80*)	p=.83 (T=-4.8, p=.02)

Table 0.2

Regional neurotransmitter concentrations per participant. Glutamate/GABA ratios are significantly lower in G.M. compared to controls. For GABA alone, there was only a trend. Cramer-Rao lower bounds (CRLBs) below 20% indicate good reliability of the acquired concentrations per region. Line widths (full-width-half-maximum, FWHM) are shown in ppm, and in brackets signal-to-noise ratio (SNR). Bold print highlights where G.M.'s value differs significantly from the controls'.

Participant	DB	JA	SF	AB	G.M.	<i>t</i> - statistic, <i>p</i> -value
IPS						
GABA						
Raw concentration (CRLB %)	1.67 (11)	.91 (22*)	0.64 (26*)	1.42 (13)	1.2 (21*)	T=.08, <i>p</i> =.94
GABA/Cr	0.222	0.152	0.096	.208	0.199	T=.46, <i>p</i> =.68
GM/WM corrected	.047	.056	.03	.068	.057	T=.38, <i>p</i> =.73
Glutamate						
Raw concentration (CRLB %)	6.5 (3)	5.39 (4)	5.07 (4)	6.89 (3)	5.5 (4)	T=-.48, <i>p</i> =.67
Glutamate/Cr	0.86	0.9	0.76	1.01	0.91	T=.24, <i>p</i> =.83
GM/WM corrected	.184	.328	.238	.33	.261	T=-.11, <i>p</i> =.92
Glu/GABA	3.88	5.91	7.95	4.87	4.58	T=-.55, <i>p</i> =.62
Linewidth (SNR)	.028 (84)	.044 (53)	.028 (87)	.028 (79)	.055 (62)	<i>p</i> =.08 (<i>p</i> =.49)

Table 0.3

IPS concentrations not used for this analysis. CRLB's exceeded the data quality control for GABA of 20% (*) in three of the five participants, including G.M.

	Numerical operations		Mathematical reasoning		Composite score	
	R	<i>p</i>	R	<i>p</i>	R	<i>p</i>
MFG						
GABA	.52	.37	.51	.38	.58	.31
Glu	-.07	.91	-.16	.8	-.07	.91
Glu/GABA	-.69	.2	-.77	.13	-.79	.11
V1						
GABA	.2	.74	.39	.52	-.36	.55
Glu	-.12	.85	-.12	.85	-.6	.29
Glu/GABA	-.36	.55	-.06	.92	-.5	.4

Table 0.4

Parametric Pearson's correlations of the standardized mathematical scores with neurochemical concentrations in the MFG and the control area (V1). None of the correlations was significant, suggesting there was no linear trend in the relationship between mathematical abilities and concentrations.

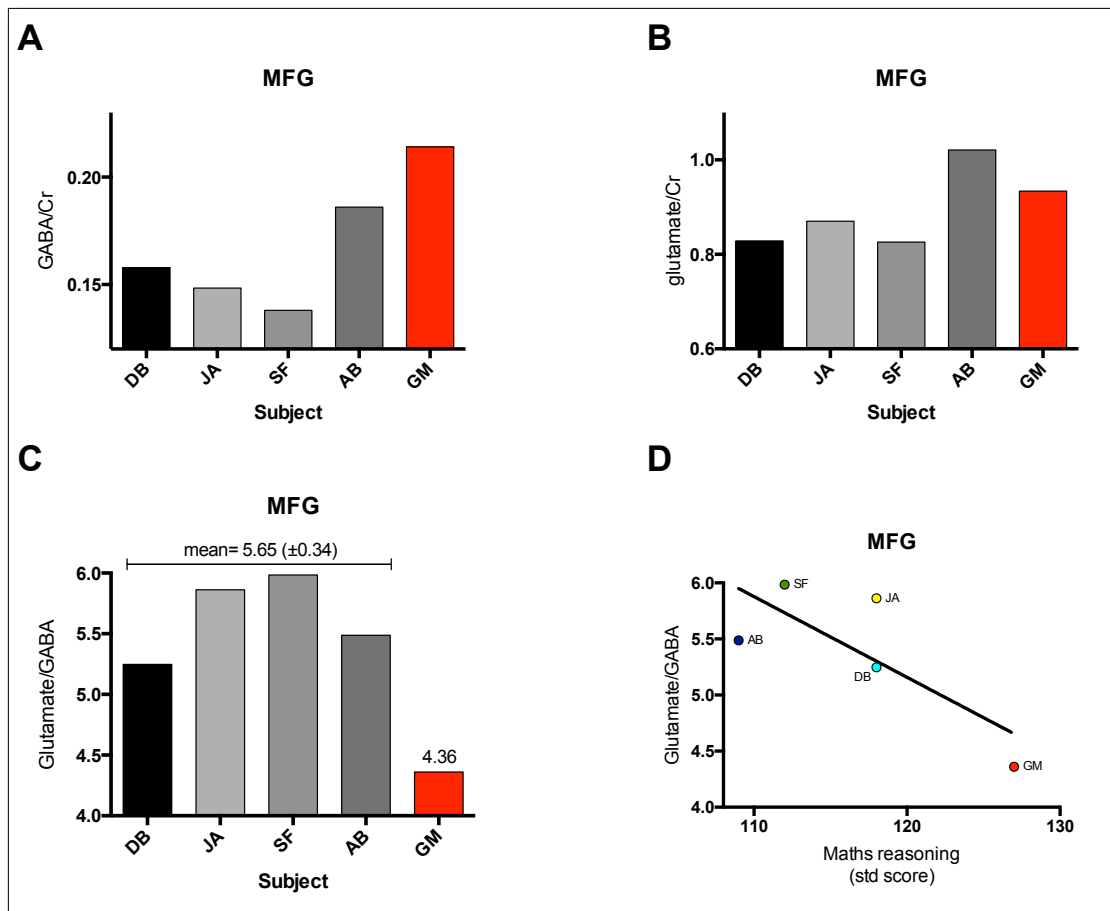


Figure 0.1

GABA and glutamate concentrations in the MFG per participant. Neither A) GABA nor B) glutamate was significantly different in G.M. compared to the controls. C) Only when investigating the ratio between glutamate/GABA, G.M.'s measures were 22.8% lower than the group average ($p=.04$, two-tailed). D) The linear relationship between the standardized mathematical reasoning scores and glutamate/GABA ratios was not significant ($p=.13$), but G.M. held an extreme value on both axes.

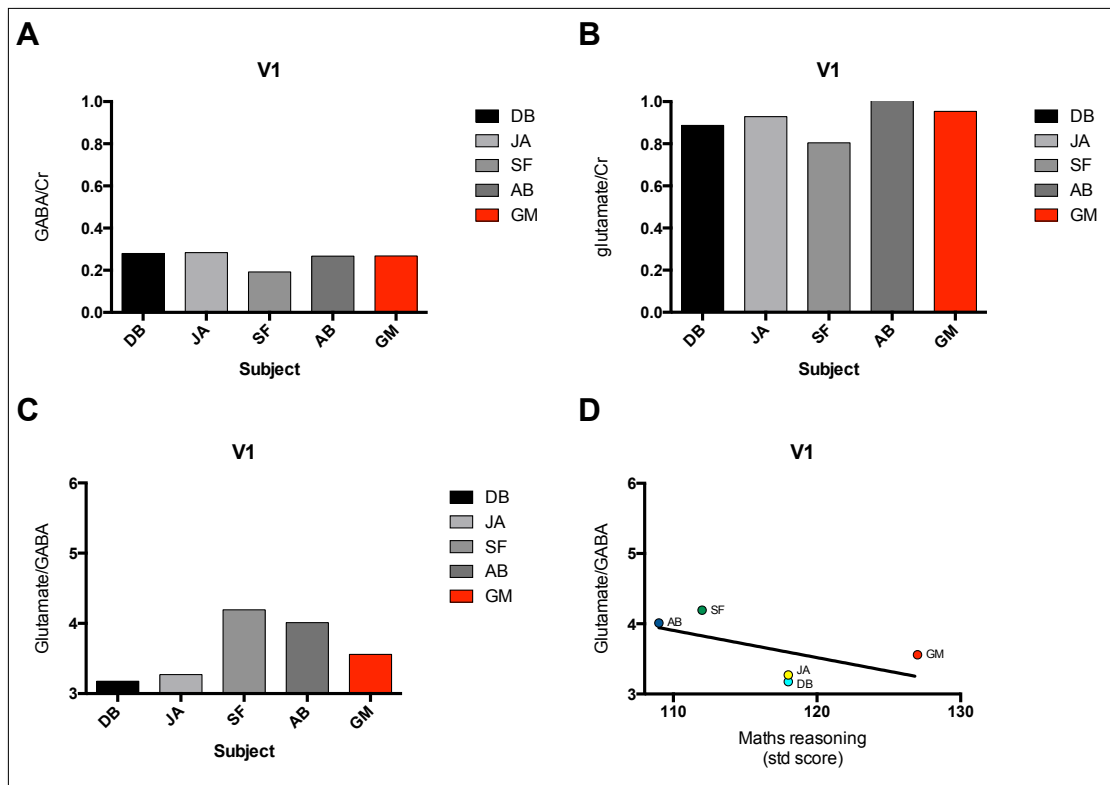


Figure 0.2

GABA and glutamate concentrations in V1. Participant SF showed lower levels of both A) GABA, B) glutamate, and C) higher glutamate/GABA ratios. G.M. showed moderate concentrations. D) Glutamate/GABA concentrations show a close to flat correlation ($p=.92$).

4.5 Discussion

In the MFG of a mental calculation prodigy, I found a reduced excitation/inhibition, as measured by non-invasive MRS glutamate/GABA ratios, in the absence of a difference in glutamate or GABA alone. Creatine, and indices for spectral quality did not differ between G.M. and the controls. G.M. was compared to four healthy, age-matched controls with high-level mathematical abilities and several years of experience in mathematical education and psychology- or neuroscience related work. In order to control for the potential of creatine to mask differences in GABA and glutamate alone, I also investigated the differences in raw metabolites, which was not significant. Accordingly, the lack of GABA and glutamate differences was independent of the effects in the reference metabolite. There were no

differences in metabolites in the intraparietal sulcus (IPS) or primary visual cortex (V1). Due to the reduced quality of GABA measurements in the IPS, as well as the relatively small sample, however, the results should be taken with care and are not conclusive. Due to the involvement of the IPS in numerical cognition (Cohen Kadosh et al., 2007; Cohen Kadosh & Walsh, 2009; Kaufmann, et al., 2008; Rosenberg-Lee, Chang, Young, Wu, & Menon, 2011), it is possible that potential effects were masked by the problems with the data quality.

My findings suggest that noninvasive E/I MRS measures in the right MFG can distinguish the highly-skilled from exceptional calculators better than concentrations of glutamate and GABA separately. Within the group, G.M.s glutamate and GABA levels were both relatively high, but not statistically significant. When combined, however, his E/I ratio was 22.8% lower than the average ratio of the four controls and still 17% lower than the lowest ratio in the sample. This apparent paradox is due to the fact that separate concentrations were scaled to creatine, whereas glutamate/GABA ratios were scaled to each other. It is surprising that superior mathematical abilities were related with *enhanced* glutamate/GABA in the child left inferior frontal gyrus in ('Inhibition, excitation, and cognitive achievement in the developing brain'). The current results suggest the opposite relationship. There are several potential explanations for this phenomenon: first, as clearly demonstrated here, there are regional differences for MRS-cognition effects and the left IFG effect was found only in children, and not adult women. It is therefore possible that the developmental effect was specific to the left IFG and may differ greatly for the right MFG. Unfortunately, there are currently no data available to investigate this question in more detail. Second, the sample in the previous study consisted of relatively normal, and not expert calculators. It is therefore possible that the relationship between E/I and cognition is not linear across different types of populations. For example, it is possible that higher cortical excitability is associated with superior mathematical abilities in normal calculators, while expert calculators may benefit from reduced excitability.

The current findings are in line with other MRS studies in healthy individuals and those with neuropsychiatric disorders, in that elevated GABA in frontal brain regions was associated with superior cognitive functioning and

performance. For example, higher frontal GABA has been associated with superior response inhibition and perseverance, reduced impulsivity, and better value-guided decision-making (Boy et al., 2011; Jocham, et al., 2012; Silveri, et al., 2013). Furthermore, only few of these studies find such associations for glutamate, but superior value-guided decision-making was additionally associated with low levels of frontal glutamate (Jocham, et al., 2012). Some few studies even report the ratios between both. One study reported higher M1 GABA, as well as GABA/gl^x⁵ ratios to be associated with superior automatic motor response suppression (Boy et al., 2010) and in a study which found higher V1 GABA, but not glutamate to be associated with reduced time perception performance, also found a trend for an inverse relationship with the glutamate/GABA ratio (Terhune, Russo, Near, Stagg, & Cohen Kadosh, 2014). Left DLPFC GABA has also been showed to first increase, and then decrease with the repeated administration of a Sternberg working memory task in normal cognition (Michels et al., 2012).

The major question in cognitive sciences on prodigies is whether the individuals' exceptional abilities are a specialized, inborn skill or whether they are acquired by extensive and prolonged practice. Cases of savants at early developmental stages have demonstrated that it is possible to solve exceptionally difficult mathematical problems, even in the absence of an understanding of even simple arithmetic (Welling, 1994). It therefore seems that the extraordinary skills of these individuals rely more upon the use of specialized and highly efficient strategies. For example, the extensive study of the male lightning calculator R. Gamm has demonstrated that his exceptional memory for numbers contributes to the quick learning of new strategies to solve complex problems, while he does not even differ significantly from controls with regard to some more simple calculations, with which he has no prior experience (Pesenti, et al., 1999). For example, Gamm reports that his mathematical performance during his school years was very poor and he never fully comprehended the concepts of arithmetic. However, his rather unusual memory for digits and numbers led him to acquire increasingly difficult calculation concepts starting at the age of twenty. His calculation

⁵ Glx is a measure of glutamate+glutamine and here I used glutamate without the influence of glutamine.

times show that the actual computation of mathematical problems takes him longer than calculations that require the more automatic retrieval of mathematical fact knowledge (Pesenti, et al., 1999). Individual patterns of brain activity during calendar calculation of another prodigy (A.B.) has also demonstrated individual use of different strategies for the performance (Fehr, et al., 2011). It is known that even non-prodigious professional mathematicians show a variety of different strategies for the same type of mathematical problems and vary in their own strategies with repeated administration of the same type of problem (Dowker, 1992). However, memory is a crucial feature of calculation abilities and from case studies it has also become apparent that a large working memory digit span (memorizing sequences of digits) is inherent in calculation prodigies (see e.g. Pesenti, et al., 1999). G.M.'s span length exceeds the standardized scoring tables and therefore lies above the 99th percentile of the population. In conclusion, it seems that a combination of extensive training and practice in the presence of already high working memory abilities contribute to the exceptional calculation abilities of prodigies. From the current study, it is difficult to distinguish the roles of learning and strategy use. G.M. himself reported that he uses strategies to simplify the problems and that everyone can learn it. However, it is unlikely that other people would achieve his calculation speed.

I suggest here that the glutamate/GABA ratio may reflect the efficiency of mathematical cognition in the right dorsolateral prefrontal cortex, and is associated with extraordinary arithmetic capacity. For firmer conclusions, larger samples of mathematically highly proficient calculators and prodigies would need to be tested.

5 Neuroenhancement of High-Level Cognition: Pushing the Boundaries of the Human Brain

Abstract

Neuroenhancement aims to improve cognitive performance in typically and atypically functioning populations. However, it is currently debated whether these are also effective in exceptionally high-functioning individuals. Theories suggest that homeostatic set points for learning and cortical plasticity limit the beneficial effects of neuroenhancement but this claim is difficult to test. To examine this hypothesis, I used transcranial random noise stimulation (tRNS) to noninvasively stimulate bilateral dorsolateral prefrontal cortices (DLPFC) of the world champion in mental calculation, G.M. TRNS did not change G.M.'s calculation performance compared to sham on an exceptionally complex arithmetic task. A control sample of calculation experts who were not prodigies (N=6) showed reduced accuracy on a complex multiplication task when stimulated with tRNS compared to sham. My findings suggest that there is an upper limit for cognitive enhancement and that an attempt to further enhance performance using tRNS may impair optimal functioning. Experimental conditions need to be carefully controlled in future studies. Such findings have scientific, societal and ethical implications about the use of brain stimulation for cognitive enhancement, as they may lead to unintended impairments in subgroups of the population.

5.1 Introduction

How far can we improve human brain functioning? Teaching and training aim to improve brain functioning and often succeed, but more recently, it has become possible to influence brain function more directly. Recent methods, such as non-invasive brain stimulation (NIBS) can lead to long-term cognitive improvements, and optimize brain functioning in normal individuals (Dayan, Censor, Buch, Sandrini, & Cohen, 2013; Krause & Cohen Kadosh, 2013). NIBS is nowadays mostly aimed to improve abilities in individuals with deficits, for instance, cognitive developmental difficulties like developmental dyscalculia or dyslexia (Iuculano & Cohen Kadosh, 2014; Krause & Cohen Kadosh, 2013; Vicario & Nitsche, 2013b). Low-performing individuals often benefit most from such neuromodulation (Sarkar, et al., 2014; Tseng et al., 2012). Since the introduction of NIBS in the research and clinical environment, we also have to consider how its use will affect different individuals, including high-functioning ones. I have previously suggested that the effects of transcranial electrical stimulation (tES) depend on individual differences in for example age, gender, brain state, and regional neurotransmitter levels (Krause & Cohen Kadosh, 2014). This means that there is the potential for individuals with extraordinary brain capacity in one or several cognitive domains to have either no gain from the stimulation, or even experience impairment. I will elaborate the problem in more detail below.

For both the recipient of the stimulation and for society, it is of high importance that we understand the consequences of NIBS in high-performing individuals. The media nowadays are promoting brain-boosting methods, especially non-pharmacological cognitive enhancement (Dresler et al., 2013). The hope is for NIBS to push the current boundaries of the human brain and cognition further than currently possible. A naturally occurring phenomenon of growing intelligence is called the Flynn effect: this is the observed gradual increase in IQ from one generation to the next (Wicherts et al., 2004). Besides this more or less natural increase in intelligence in our society, the use of NIBS may be used with the aim to artificially accelerate this progress beyond current possibilities for the supposed benefit of the individual and/or society. The consequences of such actions are unpredictable at this point, and there is

a risk of arousing unrealistic expectations. Furthermore, if NIBS impairs the abilities of some high-functioning individuals, we may unintentionally affect these peoples' future educational or occupational functioning. Cheap self-stimulation kits for the home use are already on the market (Maslen, Douglas, Cohen Kadosh, Levy, & Savulescu, 2014; Maslen, et al., 2013) and therefore there is great urgency to explore the limits of the technique in order to avoid potential abuse and protect the unskilled user from causing unwanted and possibly irreversible damage (Cohen Kadosh, et al., 2012). It is also important to educate the population about both risks and benefits of the method.

There is great enthusiasm for the use of NIBS in the enhancement of cognition, as well as the improvement of symptomatology in healthy and neuropsychiatric deficits and disorders, such as language, attention, mathematics, depression and Parkinson's disease (Benninger, et al., 2010; Demirtas-Tatlidede, Vahabzadeh-Hagh, & Pascual-Leone, 2013; Holland, et al., 2011; Snowball, et al., 2013; Weiss & Lavidor, 2012). However, current theories suggest that the effect of NIBS on cortical plasticity is limited by homeostatic set point mechanisms, such that an individual's capacity cannot be exceeded due to counter-regulation of the brain's natural balance of plasticity (Siebner et al., 2004). This means that there is an optimal level of the brain to change, but if the brain is pushed toward excessive plasticity, counter-mechanisms will kick in and avoid further changes. Consequently, no individual can progress beyond a given capacity, as mentioned above. In line with this theory, I have recently suggested that individual differences in the capacity for plasticity determine the outcome of the stimulation and that brains with high levels of plasticity are more likely to show either no effect or even impairments as a result from the stimulation (Krause & Cohen Kadosh, 2014; Krause, et al., 2013). In chapter 'The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance', I demonstrated that the right DLPFC shows high levels of excitation/inhibition ratios in an expert calculator with prodigious abilities, compared to a control group with above-average calculation abilities. It is therefore possible that such individual is equipped with close-to-boundary capacity, and therefore serves a unique participant to test this hypothesis.

Individuals with prodigious abilities have fascinated the field of psychology for decades but little is known about the underlying mechanisms of exceptional mental abilities. One of the most sophisticated of human abilities, which cannot be observed in the animal kingdom, is mental calculation (Butterworth, 1999). Calculation prodigies are characterized by extraordinary memory recall or arithmetic processing speed ('lightning calculators') and their high level of accuracy in solving highly complex mathematical problems (Snyder & Mitchell, 1999). Both structural and functional differences have been found in brain regions supporting working memory and episodic memory capacity of such individuals compared to normal calculators (Fehr, et al., 2010). In particular, the dorsolateral prefrontal cortex (DLPFC) is one of the core regions that shows elevated brain activity in a calculation prodigy (Pesenti, et al., 2001). The question arises whether we can enhance brain functioning in individuals with the highest possible abilities, which could not otherwise be learned by practice or strategy use. I examined this possibility by applying transcranial random noise stimulation (tRNS) to enhance mental calculation skills in a single case of the world's leading mental calculation prodigies (G.M.). G.M. has been practicing mental calculation since the age of three and has continuously demonstrated his outstanding abilities by winning successive world records and world championships.

I aimed to investigate the possible consequences of NIBS and hypothesized that these will lead to either of the three following options: 1) a further improvement of the already exceptional cognitive performance; 2) no effect on cognitive performance due to ceiling performance and capacity limits, or 3) impairment in cognitive performance due to the modulation of neuronal activity in a brain that already works at maximum efficiency (Krause & Cohen Kadosh, 2014). The former would provide evidence for unlimited cortical capacity or at least a wide range at the very top of the spectrum, whereas the latter option would support the homeostatic set point hypothesis. I used transcranial random noise stimulation (tRNS) to investigate this question, as this method equally stimulates two brain areas simultaneously (i.e. bilateral DLPFCs), with a high perceptual cutaneous threshold (little noticeable to the receiving participant) to ascertain proper participant-blinding (Ambrus, et al., 2010). The most likely mechanism behind the effect of the random noise has

been suggested to be stochastic resonance (Fertonani, et al., 2011). Hereby a sub-threshold stimulus (in this case weak neuronal activity) can reach the threshold when noise is added (Moss, et al., 2004). Such an effect results in an increase in synchronous neuronal firing, which may lead to an impact on cognition. For example, a recent study successfully used tRNS to bilateral DLPFCs to improve arithmetic performance in typical adult participants and the effect was still stable at a 6-month follow-up investigation (Snowball, et al., 2013).

In a second experiment (Experiment 2), I invited a small sample (N=6) of postgraduate students in the field of mathematics and statistics, who performed a complex multiplication task while receiving tRNS. Since a sample of control participants would be unable to perform G.M.'s complex calculation task, this task was designed to compare whether these highly functioning individuals would show the same direction of effects as G.M. The importance of the control sample was intended to examine the effect of NIBS on above-average, even if not prodigious, individuals.

5.2 Experiment 1: a calculation expert

5.2.1 Methods

5.2.1.1 Participant

G.M. is a male German, 46-year old, high-functioning, healthy calculation expert with no history of neurological or psychiatric conditions. He has been engaging in competitive mental calculation events for more than 25 years and is a member of Mensa, The High IQ Society. G.M.'s exceptional mental calculation skills have repeatedly been demonstrated in international mental calculation competitions such as the Mental Calculation World Cup or the Mind Sports Olympiad (MSO). G.M. is a nine-fold winner of the MSO mental calculation gold medal, and he holds several world records in mental calculation. He holds two doctorate university degrees in humanities and is also highly skilled in calendrical calculation. I measured his standardized mathematical abilities to be above the 99.8th percentile (top composite standard score = 143, WIAT-II-UK, see 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise

stimulation; Methods; Standardized mathematical assessment'), and also in measurements of fluid reasoning and mental speed he scored above the 99.9th percentile, resulting in an extrapolated IQ estimation of above 160 (see 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation; Standardized mathematical assessment' and '5.2.1.2: Standardized batteries'). Written informed consent was received from G.M. prior to the beginning of the study. The study was approved by the Berkshire Research Ethics Committee.

5.2.1.2 Standardized batteries

I assessed G.M.'s basic mathematical abilities prior to the stimulation experiment (see 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation, Standardized mathematical assessment'). Fluid reasoning was assessed using the Culture Fair Intelligence Test, which eliminates potential cultural influences on intelligence measurements (CFT-20R; Weiß & Weiß, 2006). As G.M. exceeded the standardized scores of IQ measures, his IQ was estimated to be above 160. Furthermore, G.M.'s mental speed was measured using a trail-making test (ZVT; Oswald & Roth, 1987). Again, G.M. exceeded the standardized scores, with an extrapolated IQ estimate above 160⁶.

5.2.1.3 Stimulation task

The task consisted of highly complex calculations that could not be performed by individuals with normal, or even advanced calculation skills. The problems presented on a computer screen involved either a 100- or a 120-digit number that had to be broken down into 20 different 6-digit prime number factors that, multiplied by each other successively, made up the presented number (i.e. prime factor 1 * prime factor 2 * [...] * prime factor 20 equaled this large number). G.M. had to either successfully identify one of these twenty prime numbers, or additionally provide the factor position within the multiplication

⁶ These test scores (CFT-20R and ZVT) were kindly provided by my collaborator Dr Martin Dresler and mentioned here in order to demonstrate that G.M. is very high functioning even outside his domain of expertise. The scores were used in the submitted manuscript.

sequence (1-20) (Figure 5.1). During the entire task execution, G.M. had lists of all prime numbers up to 1,000,000 in front of him in order to confirm that his final answer comprised an existing prime number. It is important to note that despite the fact that some individuals have the prodigious ability to recognize or produce large prime numbers while being incapable of performing mental arithmetic (Welling, 1994), G.M.'s expertise is specifically in fast mental calculation and he has not memorized all 78,499 prime numbers between 1 and 1 million. Note that in such a rare single-case study, it is impossible to have a control group that would be able to solve the same task problems. Therefore, the best practice in this case is to use the participant as his own control (Cohen Kadosh, Tzelgov, & Henik, 2008; Sapir, Soroker, Berger, & Henik, 1999).

Generate new number

724166410115167386070961764994504504745380659954527299625192155421665921543526853479203656129176378918205421850670419312829

Prime factor: 984007 Position: 11 Check Correct!

1	2	3	4	5	6	7	8	9	10
983849	983861	983863	983881	983911	983923	983929	983951	983987	983993
11	12	13	14	15	16	17	18	19	20
984007	934017	984037	984047	984059	984083	984091	984119	984121	984127

Figure 5.1

G.M.'s calculation task: a 120-digit number (top) was generated on button click. This number was the product of twenty successively multiplied 6-digit prime numbers (positions 1-20 displayed here). One of these had to be identified and entered as quickly as possible. In more complex trials, the exact

position of the prime factor in the succession of multiplications additionally had to be identified. The task provided feedback on the correctness of the response.

5.2.1.4 Stimulation experiment 1

On two consecutive days, G.M. underwent tRNS (0.1-500Hz frequency range⁷) in six double-blind sessions using a wireless Starstim Neuroelectronics® stimulator (Barcelona, Spain). TRNS and sham stimulation were applied in a randomised order. The current (1mA) was delivered by two circular electrodes of 25cm² each to F3 and F4 electrode positions, according to the international 10-20 system for EEG recording. TRNS was applied in eight sessions across two days during the administration of the calculation task for 20 minutes per session with 15 seconds ramp up and ramp down. Sham stimulation consisted of 30 seconds of stimulation with 15 seconds ramp up and ramp down to mimic the skin sensations experienced during real stimulation and thereby ascertain proper participant-blinding. Due to a crash of the software, the first session (tRNS) terminated after a few minutes and the data were discarded. After a short break, the session was resumed with the same stimulation parameters. By the beginning of the 8th session, G.M. reported severe concentration problems, such that the session was also terminated. Accordingly, 6 full sessions of data were available for analysis. G.M. reported no skin sensations under the electrodes during any of the sessions, confirming the higher cutaneous perception threshold compared to other forms of electrical stimulation (Ambrus, et al., 2010). G.M. was also unable to guess the stimulation conditions in all cases. The eventual task order was tRNS, sham, tRNS, sham, sham, tRNS.

5.2.2 Results

During each session, G.M. performed complex calculations at four different levels of difficulty. The order of levels of difficulty was randomized across

⁷Note that at the time of the experiment, Neuroelectronics® did not provide the options to modify the frequency of tRNS, but it was important for the participant of the study to move around freely. Therefore, the stimulation settings are slightly different in this compared to the other studies in this thesis.

sessions. Trials that were answered incorrectly were excluded from the analysis (8.6% with no significant differences between the number of trials under real (N=4) and sham (N=5); Fisher's exact test, $p>.2$). Response times from problem presentation until logging in of G.M.'s answers were recorded by two experimenters. One trial was excluded from the analysis due to an accidental difference of 50 seconds between the observers, and was attributed to an experimental error. Scores were computed for each trial as the average times from the two results. The correlation between the observers recorded time was .99 (Spearman's R, $t(53)=95.02$, $p<.001$). Outliers were removed if calculation times exceeded more than 2.5 standard deviations of the mean. For inferential statistics, each trial was considered a case, resulting in 76 trials in total. TRNS and sham calculation times were not correlated ($r_p=-.24$, $p=.15$). Paired-samples or repeated measures tests were not justified due to the unequal numbers of trials per condition (sham N=41, tRNS N=35). A one-way analysis of variance (ANOVA) demonstrated that sham and tRNS did not differ in their calculation times ($F(1,74)=.35$, $p=.56$). The result was the same using the non-parametric related-samples Wilcoxon signed rank test ($W(35)=5,344.5$, $Z=25$, $p=.8$). A Wilcoxon signed-rank test on accuracy on the three sessions per condition revealed no significant difference either ($W<.001$, $Z=-1.63$, $p=.1$).

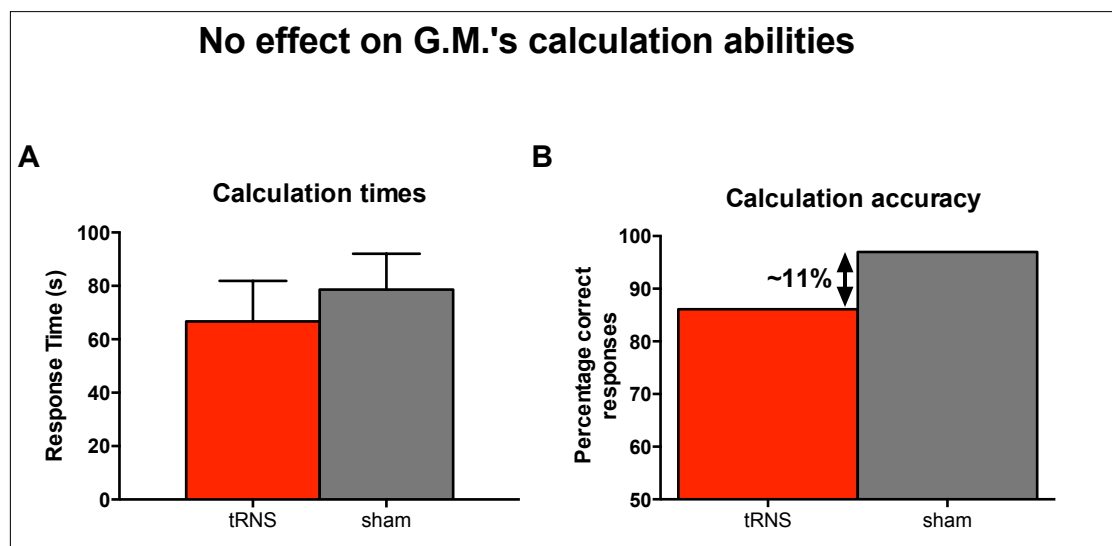


Figure 5.2

G.M.'s calculation performance during tRNS stimulation and sham control. A) There was no effect of stimulation on A) calculation times in seconds or B) accuracy (in percent). Accuracy was 10.9% higher in the sham condition, which was not significant (related-samples Wilcoxon rank test).

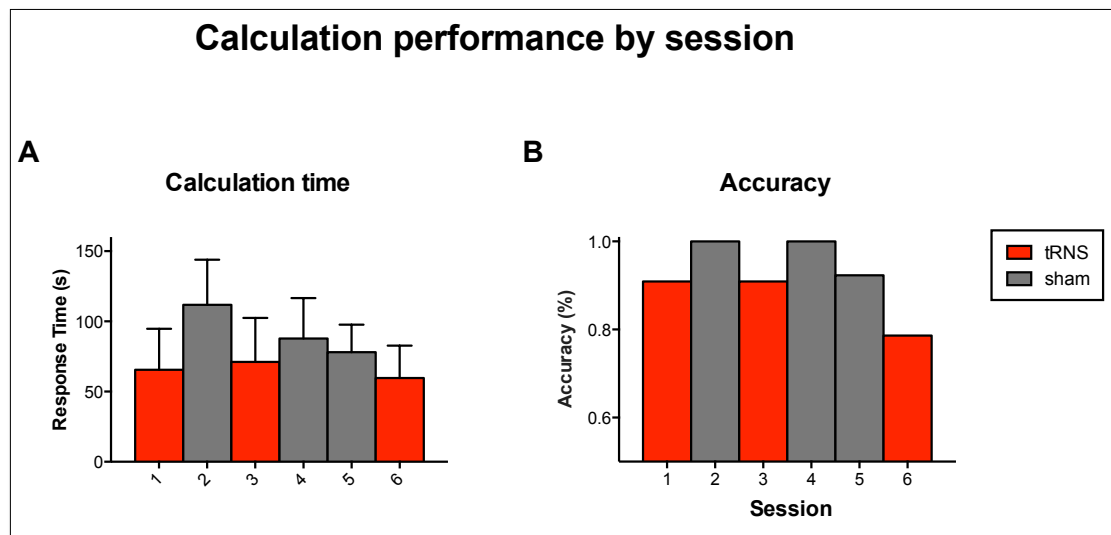


Figure 5.3

G.M.'s calculation performance across stimulation sessions. His calculation time improved, and accuracy decreased towards the end of the experiment. Sessions 1-3 occurred on day 1 and sessions 4-6 on day 2. A speed-accuracy trade-off is possible.

5.2.3 Intermediate discussion

Using a type of NIBS that is known to increase neuronal excitability both during and after the cessation of the stimulation (Antal et al., 2008; Terney, et al., 2008), tRNS to bilateral DLPFCs of G.M. did not affect calculation times. Accuracy decreased by almost 11% under tRNS compared to sham, but the difference was not significant. It is also important to note that the reduction in accuracy was accompanied by an improvement in calculation times. Therefore a speed-accuracy trade-off may have developed during the second day of stimulation. Unfortunately, G.M. was not available for further sessions on other days, which could have improved the control over experimental testing conditions. This was a single-case study with a unique participant, which caused difficulties with data acquisition, statistical analysis and

subsequent interpretation of the results. Therefore, it is difficult to draw firm conclusions on these observations. G.M. cannot be compared to normal, or even very proficient calculators, based on the complexity of the task at hand. In order to investigate further, how tRNS affects individuals with high arithmetic abilities, I tested an additional sample of 6 healthy postgraduate students with similarly high standardized mathematics scores.

5.3 Experiment 2: proficient calculators (controls)

5.3.1 Methods

5.3.1.1 Participants

Six healthy postgraduate students in the field of mathematics and statistics from the University of Oxford were recruited to represent a mathematically highly proficient sample (details see Table 5.1⁸). Written informed consent was acquired before the beginning of the first testing session. They were financially compensated for their time and effort. The study was approved by the Berkshire Research Ethics Committee.

Participant	Age	Numerical Operations		Mathematical Reasoning		Composite Mathematics	
		Std	(%)	Std	(%)	Std	(%)
G.M.	46	130	(98)	127	(96)	143	(99.8)
1	23	123	(92)	114	(94)	121	(92)
2	30	126	(96)	126	(96)	136	(99)
3	25	121	(92)	126	(96)	131	(98)
4	35	128	(97)	123	(94)	135	(99)
5	30	122	(93)	111	(77)	118	(88)
6	25	115	(84)	102	(55)	107	(68)

⁸ Please note that the age-matched control participants from the chapter ‘The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance’ were senior staff of the university and either not willing or not eligible to receive electrical stimulation. On the contrary, I did not scan the control participants from this study due to large age gap with G.M. GABA and glutamate have been shown to underlie ageing effects (Chang, Jiang, & Ernst, 2009; Gao et al., 2013).

Table 5.1

Participants' baseline mathematical abilities, including G.M. In order to avoid recognizability of the individual participants, the field and participant of study are not included in the table. The standard scores (Std) and percentiles (in brackets) show that despite almost ceiling-level performance in the controls, G.M. had the highest possible score on all scales.

5.3.1.2 Calculation task

Since G.M.'s task was considered impossible to solve for normal participants, even if highly proficient in mental arithmetic, I designed a simplified version that assessed accuracy and calculation speed. The task consisted of 40 multiplication problems with 4 different subtypes to mimic differences in the use of strategies of G.M.'s task. The 4 problem types were either multiplying a 2-digit by a 2-digit number (e.g. 34×76); a 3-digit by a 2-digit number (e.g. 669×86); a 3-digit by a 1-digit number (e.g. 539×7); or a 4-digit by a 1-digit number (e.g. 3746×7). The problems were controlled for shortcuts by excluding units of '2', '5' or '0', or same units (e.g. 36×76). The task was presented with white letters on a black background on a computer screen. The participant had to manually enter the answer into a response window and two experimenters took the times using stopwatches on each trial. There were two different sets of problems (version A and B), one for each session. None of the problems occurred twice across versions but the type of problems was the same. The interacting experimenter was blind to the stimulation condition and the stimulating experimenter (myself) noted the time when the stimulation ended. The task lasted beyond the stimulation duration in all cases. For one participant, the non-stimulation experimenter had to leave the room due to an unusually long task duration, and therefore 12 measurements were missing. Participants were given the opportunity to rest for a minute in between trials, as some participants experienced fatigue throughout the task.

5.3.1.3 Stimulation experiment 2

Stimulation settings were as described in Stimulation experiment 1.

5.3.1.4 Procedure

Participants came to a first testing session, in which their basic mathematical abilities were assessed (WIAT[®]-II-UK; Wechsler, 2005). As in G.M.'s case, the stimulation sessions took place on two different days (sessions 2 and 3), with a single stimulation session each. After attaching the electrodes and setting the stimulation parameters, participants started with the first calculation problem and had unlimited time to respond and enter each answer by hand. The duration of the task varied with the participants' calculation speed. Participants received the task problem sets either in the order A/B or B/A for sessions 2/3.

5.3.2 Results

There were no differences in standard scores between G.M. and controls using Crawford's modified t-test (see 'The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance, Crawford's modified t-test for single case studies'; Crawford & Howell, 1998; numerical operations: $t(5)=1.54$, $p=.18$; mathematical reasoning: $t(5)=.96$, $p=.38$; composite score: $t(5)=1.49$, $p=.2$). In the control group, there was no significant difference between the number of correctly answered items within the tRNS and after the tRNS period ($W=12.5$, $Z=.42$, $p=.67$), using the related-samples Wilcoxon signed-rank test. Similarly, the difference in accuracy (percentage of correct responses) within and after tRNS was not significant either ($W=14$, $Z=.73$, $p=.46$). Overall however, the number of correctly answered problems was significantly higher in the sham compared to the tRNS condition ($W=21$, $Z=2.21$, $p=.03$) and similarly the percentage of correct responses (accuracy) ($W=20$, $Z=1.99$, $p=.046$) (Figure 5.4 B). Participants performed 45% better in the sham (mean=68.33, SD=28.75) than the tRNS condition (mean=37.5; SD=18.3). When participant 5 and 6 were removed due to their lower baseline mathematical abilities, there was still a trend for the error rate (accuracy in percent: $W=9$, $Z=1.46$, $p=.14$; number of correctly answered items: $W=10$, $Z=1.83$, $p=.07$).

When entering all response times for correct responses into a repeated measures ANOVA with condition (tRNS/sham) as the within-subject factor, the conditions were not significantly different from each other ($F(1,143)=.25$,

$p=.62$, $\eta_p^2=.002$; $p=.76$ with subject-specific outliers removed). This was confirmed using the non-parametric related-samples Wilcoxon test with all trials included ($W=5,344.5$, $Z=.25$, $p=.8$). Since participants 5 and 6 showed lower baseline abilities in mathematics, I repeated the analysis with the other 4 participants only, which did not change the result ($F(1,89)=1.31$, $p=.26$, $\eta_p^2=.01$; non-parametric $W=2,058$, $Z=-.04$, $p=.97$) (Figure 5.5).

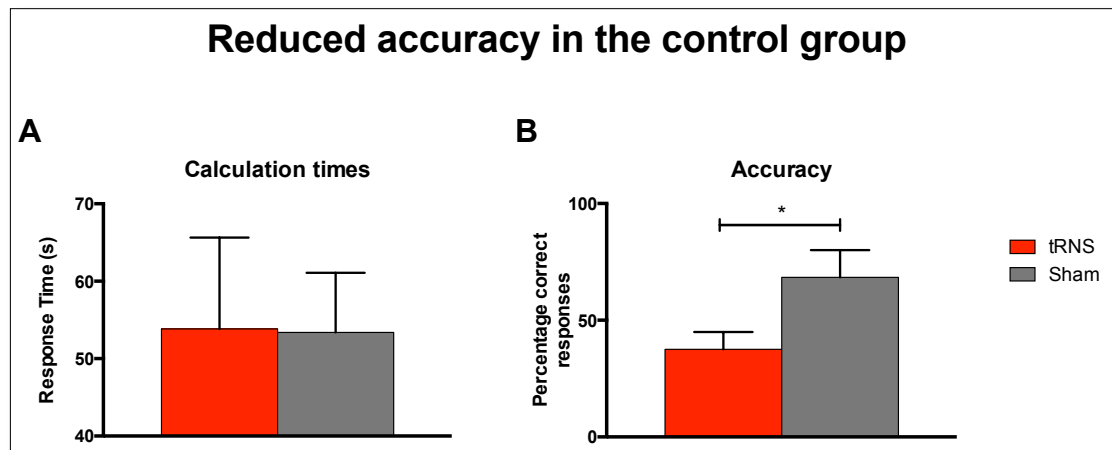


Figure 5.4

Performance of the whole participant group per condition (here means and SEM). A) Response times were not significantly different from each other, whereas B) accuracy in terms of the percentage of correctly answered items (out of 40) was significantly higher (45%) in the sham compared to the real tRNS ($p < .01^{**}$).

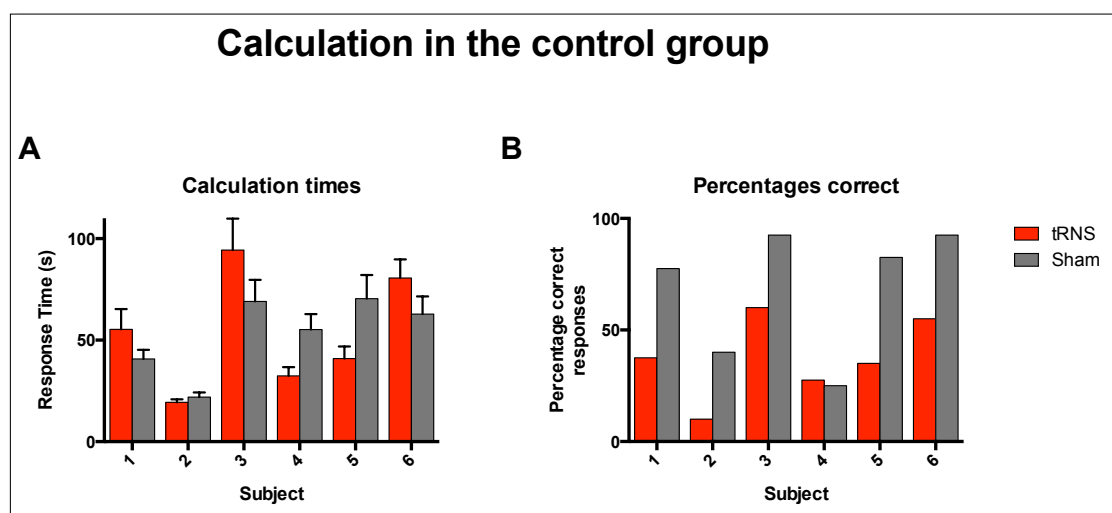


Figure 5.5

Calculation times on the correctly answered multiplication problems by participant. A) While participant 1, 3, and 6 were faster under sham stimulation, participants 2, 4, and 5 were faster under tRNS. This effect cannot fully be explained by the counter-balanced order of calculation sheets, since participants 1,3, and 5 had the same order, and participants 2,4, and 6. B) Accuracy in terms of the percentage of correctly answered items. Participant 4 was the only participant in whom accuracy was similar under tRNS and sham, but accuracy was generally fairly low for this participant.

5.3.2.1 Correlations

Non-parametric Spearman correlation coefficients were computed for baseline (WIAT-II-UK subtest standard scores) and the difference between response times and accuracy under sham and real tRNS (sham – tRNS = difference). The directions of correlation coefficients were unspecific and all $p>.17$, except for a trend between composite score and accuracy difference ($p=.07$). The correlation suggested that higher baseline mathematical abilities were associated with a larger impairment under tRNS.

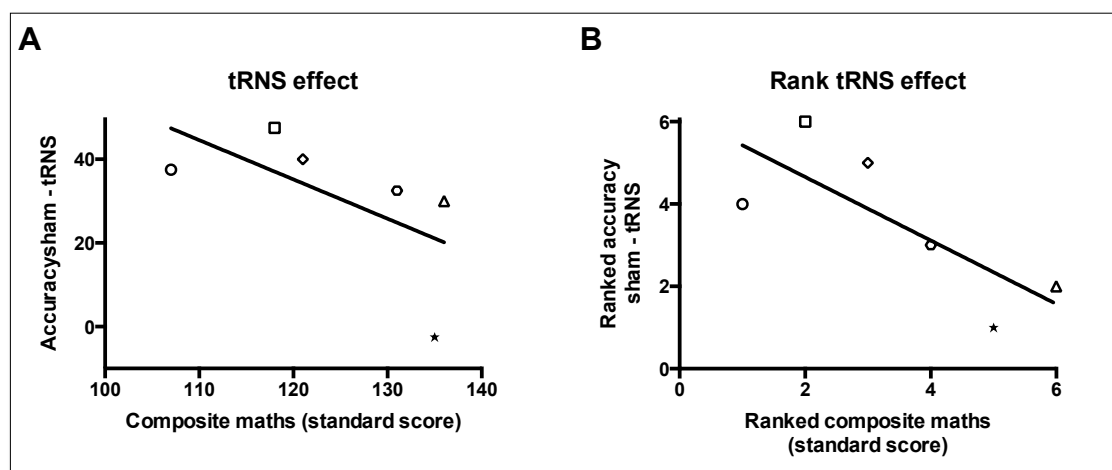


Figure 5.6

Correlations between baseline mathematical abilities and the change in accuracy between tRNS and sham. A) The Pearson's correlation coefficient was not significant ($r=-.61$, $p=.2$). However, participant 4 (★) represented an outlier due to the low accuracy and a small difference between tRNS and sham. B) Non-parametric Spearman correlations showed a trend toward a

correlation between the tRNS effect and baseline mathematical abilities ($r_p = -.77$, $p = .07$). The small sample size, however, does not allow for clear interpretations.

5.4 Discussion

TRNS to bilateral DLPFCs did not modulate calculation performance in a calculation expert with prodigious abilities. An 11% decrease in accuracy, co-occurred with a slight improvement in calculation times, which seemed due to a speed-accuracy trade-off. The changes in calculation times and accuracy under tRNS compared to sham were, however, not significant. The control participants also showed a close to 31% impairment in accuracy under tRNS compared to sham, but no change in response times. While it is hard to interpret the results from G.M. alone, together these results from all the participants together support the idea that the use of tRNS in individuals who are already performing at a high level may have an effect opposite to the intended effect, and may therefore an attempt to further improve extraordinary performance by tRNS can compromise existing cognitive abilities (as implied by the set point theory). However, the sample size and relatively low control of testing conditions, however, limit the generalizability of the present effects.

To a certain degree, the current findings mirror results from a tDCS study, in which both expert and novice pianists underwent piano key stroke sequence training during a 15 minute bilateral anodal-cathodal electrode configuration to primary motor cortices (Furuya, Klaus, Nitsche, Paulus, & Altenmueller, 2014). Pianists (all majored in piano music and had been training on the piano extensively for at least 13 years) deteriorated under the same conditions but not when anode and cathode were reversed. The authors of this work also examined novices, who showed gains in fine motor control in both left and right hands under anodal tDCS to the contralateral, and cathodal tDCS to ipsilateral cortices, compared to sham. The authors hypothesized that the effect of tDCS here depended on the initial expertise and that an inverted-U shape may indeed have led to this decrease in fine motor abilities in those who already performed highly.

It should be considered that G.M.'s or even the control participants' level of ability is unlikely to be reached by the majority of people. In contrast, previous studies by our lab have demonstrated improvements in complex calculation in university students using tRNS settings similar to those used here (Snowball, et al., 2013; 'Inhibition, excitation, and cognitive achievement in the developing brain'). Even though several studies have found no results or even impairments for numerical or mathematical abilities under certain stimulation settings of tDCS to bilateral PPCs, there was less evidence for impairments using DLPFC stimulation, and tRNS has not been found to cause such impairments yet (Cappelletti, et al., 2013; Cohen Kadosh, Soskic et al., 2010; Hauser, et al., 2013; Luculano & Cohen Kadosh, 2013; Rutsche, et al., 2015; Sarkar, et al., 2014). It is also possible that the higher range of noise frequency acts on different mechanisms than high-frequency stimulation and thereby causes oppositional effects (Saiote, et al., 2013).

It is also important to consider that most NIBS studies are based on above-average intelligent university students. If individual tailoring of the stimulation is deemed necessary, future studies need to be performed on more heterogeneous samples that reflect the part of the population that qualifies as the clinical target. It would also be desirable to measure glutamate and GABA concentrations before and after stimulation in the same participant group, which, unfortunately, was not possible in the current thesis work. Therefore, it is currently difficult to make broad predictions. Based on the present findings, I suggest that there is a limit to cognitive enhancement using NIBS techniques, at least in the highly skilled. Further investigations of larger and more heterogeneous samples are required to draw firm conclusions on this topic and the consideration of various individual differences would be valuable to account for.

The current study unfortunately lacked the possibility to compare G.M. to a suitable control group of normal, healthy volunteers who could be administered the same task. Despite the fact that I chose a sample of mathematically highly proficient controls, neither the arithmetic abilities, nor the tasks were comparable, which complicates the comparability of the effects found here. Ideally, in order to directly compare participants, G.M. would perform on the same task, which can be corrected for ceiling effects: for

example a study similar to the developmental dyscalculia study by luculano and Cohen Kadosh, in which two participants with below-average numerical abilities underwent numerical training under opposite parietal anodal-cathodal tDCS (luculano & Cohen Kadosh, 2014). Since the paradigm had been tested in proficient calculators earlier (luculano & Cohen Kadosh, 2013), it could be concluded here that one dyscalculia participant performed contrary to what was expected from the normal group when stimulated with similar parameters. In sum, while other studies have found that participants with the lowest initial abilities gain most from the stimulation (Sarkar, et al., 2014; Tseng, et al., 2012), the current results provide further support for the homeostatic set point hypothesis of cortical excitability, in which no further improvement, or even impairments in abilities are found upon the induction of accumulated increases in cortical excitability (Krause, et al., 2013; Siebner, et al., 2004). I was therefore able to provide preliminary evidence that individuals with high expertise in mathematics show impairments under tRNS, when compared to sham. The consequences of introducing methods for neuroenhancement on the society are currently unpredictable but require serious discussion and regulation (Maslen, et al., 2014), especially as they might be associated with costs for the individual (luculano & Cohen Kadosh, 2013; Sarkar, et al., 2014). Ideally, the results will trigger further research to examine whether the NIBS user and the application to different individuals should be considered more carefully than previously assumed. It is a dangerous leap from anticipating physiological changes induced by the method to predicting the behavioral outcomes and the relationship between these effects should not be taken for granted (Bestmann, et al., 2015). As such, the current results are important for psychologists, neuroscientists, and ethicists, as they raise societal and neuroethical concerns akin to doping in athletes and attempting to increase the disparity in mental abilities in the general population.

6 Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation

Abstract

Learning and memory are associated with altered transmission of gamma-aminobutyric acid (GABA) and glutamate, the brain's major inhibitory and excitatory neurotransmitters, respectively. Basic motor learning studies have demonstrated that transcranial direct current stimulation (tDCS) can modulate GABA and glutamate and improve learning. Here I used an established training paradigm to investigate how transcranial random noise stimulation (tRNS) affects regional concentrations of GABA and glutamate associated with complex arithmetic learning. In a between-subject, double-blind, sham-controlled study, seventy-two participants underwent tRNS to either dorsolateral prefrontal cortices (DLPFCs), posterior parietal cortices (PPCs) or sham. I assessed glutamate and GABA both before and after the completion of the training. In addition to the tRNS effects on arithmetic training and neurochemicals, I also performed a correlational analysis of a sub-part of the sample involving the left IPS at baseline and standardized mathematical abilities. I compared these correlations with those in the chapter 'Inhibition, excitation, and cognitive achievement in the developing brain', which involved the IPS in the right hemisphere. While there were no associations in the right IPS previously, I now found positive correlations between glutamate, as well as GABA and mathematics, and a negative correlation for the glutamate/GABA ratio. This result suggests that there may be hemispheric differences in the role of glutamate and GABA in tasks used in such standardized tests. Furthermore, I found no cognitive improvements under DLPFC tRNS, but impairments in calculation training and reduced calculation performance in a subsequent task presenting both trained and untrained material in the PPC tRNS group. At follow-up, there was no longer evidence of impairments. There was a general reduction in creatine, the reference

metabolite for glutamate and GABA, across the training period, but no tRNS effects on neurochemicals could further be found. It is possible that the impairment due to PPC tRNS was too subtle and short-lived to cause changes in glutamate and/or GABA. Sample-specific effects may have led to the behavioral results but great caution is recommended, as the observed impairment in calculation under parietal tRNS has the potential to affect cognitive performance in real life.

6.1 Introduction

Both glutamate and GABA, the brain's major excitatory and inhibitory neurotransmitters, respectively, have important roles in learning and brain plasticity (Ge & Dani, 2005; Trepel & Racine, 2000). Using noninvasive magnetic resonance spectroscopy (MRS), various studies have found associations between regional concentrations of glutamate and/or GABA and complex cognitive abilities. For example, reduced GABA in frontal regions has been associated with higher impulsivity, lower response inhibition, and a lack of perseverance, and value-guided decision-making (Boy, et al., 2011; Jocham, et al., 2012; Silveri, et al., 2013). On the contrary, superior performance in value-guided decision-making was associated with higher levels of glutamate (Jocham, et al., 2012). While higher GABA concentrations in the frontal cortex are often associated with better cognition in both the healthy brain and in neuropsychiatric disorders, the relationship in other brain areas is often reversed. For example, frontal glutamate was higher in treatment-resistant patients, and correlated positively with attention performance in patients with schizophrenia (Rowland, et al., 2012; Szulc et al., 2013).

In the primary visual cortex (V1), some higher-level cognitive abilities are also associated with increased GABA, such as the frequency of daily life cognitive failures (Sandberg et al., 2014), smaller impairments in visual inhibition in schizophrenia (Yoon, et al., 2010). However, increased GABA was also associated with poorer abilities in time perception and increased sleeping problems in insomnia (Morgan et al., 2012; Terhune, et al., 2014). Even though most of these findings involved only GABA, but not glutamate, some

few studies have also found cognitive effects for glutamate in V1. Namely, increased glutamate in V1 has been associated with poor reading abilities in children (Pugh et al., 2014), but also facilitated perception of phosphenes induced by transcranial magnetic stimulation (TMS) (Terhune et al., 2015).

In M1, baseline GABA was positively associated with motor response times and smaller automatic motor responses, such that higher GABA was related with slower motor performance and enhanced suppression of involuntary priming-evoked motor activation (Boy, et al., 2010; Stagg, et al., 2011). Notably, regional concentrations of GABA and glutamate can change with learning, such that a short period of motor learning can reduce GABA in the left primary motor cortex (M1) significantly (Floyer-Lea, et al., 2006). Similarly, M1 GABA has been showed to decrease after several weeks of low-intensity juggling practice (another form of motor learning), and has reverted back to the baseline six weeks after cessation of the training (Sampaio-Baptista et al., 2015).

One of the pioneer studies using transcranial direct current stimulation (tDCS) found that excitatory A-tDCS over left M1 at rest reduced GABA, while inhibitory C-tDCS reduced glutamate and GABA (Stagg et al., 2009). Here, the change in glutamate was positively associated with change in GABA. A-tDCS was also found to decrease M1 GABA, while improving performance and retention in motor memory, but no changes were found for glutamate (Kim, et al., 2014). Also in the parietal cortex, A-tDCS could enhance glutamate levels (Clark, et al., 2011). The initial relationship between GABA and motor response times could be modulated with A-tDCS and the induced reductions in GABA (in percent change) was correlated with the improvement in motor learning (Stagg, et al., 2011).

Transcranial random noise stimulation (tRNS) is an excitatory, noninvasive brain stimulation technique, which uses a rapidly alternating current at a fixed range of frequencies to induce cortical excitation in the brain area under both electrodes (which functions as anode and cathode in tDCS) (Terney, et al., 2008). TRNS has the potential to modulate higher-order cognitive performance, as well as brain metabolic functioning, and can enhance brain plasticity for learning (Fertonani, et al., 2011; Saiote, et al., 2013; Snowball, et al., 2013). Several cognitive studies have documented effects of tRNS on

cognition, including numerical and mathematical abilities (Ambrus et al., 2011; Cappelletti, et al., 2013; Mulquiney, et al., 2011; Snowball, et al., 2013). Some of these studies found positive results, whereas others report impairments in cognition under certain conditions. I therefore investigated whether tRNS can modulate complex arithmetic training and transfer effects, as well as the associated suggested markers for learning and plasticity. The latter was assessed by quantifying GABA and glutamate concentrations before and after the training and stimulation using MRS. I have previously demonstrated that tRNS to bilateral DLPFC and PPC can improve complex arithmetic learning, but had no effect in a calculator with prodigious abilities, despite his atypical prefrontal excitation/inhibition balance ('Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation', 'The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance', 'Neuroenhancement of High-Level Cognition: Pushing the Boundaries of the Human Brain'). Specifically, I have previously demonstrated that complex arithmetic training can be improved using bilateral prefrontal and parietal tRNS even in a small sample of proficient calculators and under repeated tRNS administration ('Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation'). These results partly confirmed the findings from another study performed in our lab (Snowball, et al., 2013). The original training task (without the use of stimulation) has been found to modulate brain activity in both frontal and parietal brain areas (Delazer et al., 2005). In the current study I investigated the effect of tRNS on regional concentrations of GABA and glutamate in the stimulated brain area, and their link to cognitive training and transfer. I therefore tested 72 proficient calculators undergoing five consecutive days of complex arithmetic training and brain stimulation, under bilateral DLPFC, PPC, or sham tRNS. I scanned the participants before and immediately after the training period to investigate the changes in GABA and glutamate in the stimulated brain areas. Furthermore, in the chapter 'Inhibition, excitation, and cognitive achievement in the developing brain', I showed that there was no relationship between glutamate and GABA and mathematical abilities in the right IPS of adults. I suggested in that chapter that the link might be observed in the left parietal cortex. Here I additionally

performed correlations between mathematics and glutamate and GABA in the left IPS in order to investigate whether there were hemispheric differences in neurochemical-behavior relationships.

6.2 Methods

6.2.1 Participants

72 healthy, right-handed participants were recruited for the current study (age, 36 males (mean age 21.75, SD=0.54, range 18-30), 36 females (mean=22.1, SD=0.44, range 19-28)). All met the safety criteria for tES and MRI, were medication free and had no history of psychiatric or neurological disease. Participants were compensated for their time and effort with £7.50 per hour of behavioral testing and scanning, and with £10 per hour for tRNS sessions.

	Male	Female
Parietal tRNS (N=24)	21.75(.96)	22.08(.79)
Parietal Sham (N=12)	21.17(.98)	21.33(1.41)
Frontal tRNS (N=24)	22.33(1.2)	22.08(.7)
Frontal Sham (N=12)	21.17(.6)	23(.93)

Table 6.0.1

Mean age and standard deviations (in brackets) per stimulation group by gender. The groups did not differ significantly in age (group x gender interaction: $F(3,64)=.32$, $p=.81$).

	Guessed sham (N=12)	Guessed real (N=24)	Total recorded
Parietal tRNS	14	6*	20/24
Parietal Sham	7*	3	10/12
Frontal tRNS	17	5*	22/24
Frontal Sham	6*	4	10/12

Table 6.0.2

Frequencies of recorded guesses of whether participants thought they received real or sham stimulation. *= guessed correctly. 13 of the 20 recorded responses in the two sham groups were correctly identified as sham, whereas only 6 out of 20 in the parietal, and 5 out of the 22 in the frontal group identified real stimulation correctly. This analysis indicates that tRNS to the parietal and dorsolateral prefrontal cortices were not consistently noticeable and participant blinding was successful, which is in line with previous findings on tRNS to the motor cortex (Ambrus, et al., 2010).

6.2.2 Standardized mathematical assessment

I assessed basic mathematical abilities at baseline (see ‘Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation, Standardized mathematical assessment’).

6.2.3 Training paradigm

See ‘Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation; Methods; Complex arithmetic training’.

6.2.4 MRS

For the current study, I placed voxels in the left middle frontal gyrus (MFG) for participants in the DLPFC tRNS or sham group, and in the left intraparietal sulcus (IPS) for those in the PPC tRNS or sham group (see chapter ‘Inhibition, excitation, and cognitive achievement in the developing brain’). Note that due to time limitations, I scanned the DLPFC in half of the sham group (MFG, N=12), and the PPC in the other half (PPC, N=12). V1 as a control VOI was additionally scanned for all participants (N=72). Glutamate and GABA concentrations are reported after correcting for gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) voxel contributions.

6.2.5 Procedure

Participants were screened for safety over the phone and invited to the Centre for Functional MRI of the Brain (FMRIB) at the University of Oxford. A one-hour scan (localizer, structural MRI, spectroscopy of two VOIs, starting with V1, then followed by either MFG or IPS) was performed and the participant was subsequently invited to the department of experimental psychology for five days of tRNS and training. The interval between the scan and the first session was between one day and three weeks, due to difficulties with the scanner bookings. On the Monday, basic mathematical abilities were assessed using the WIAT-II UK. Head measurements were taken and the electrodes were attached to either F3-F4 or P3-P4. A second experimenter started the stimulator to ascertain adequate double-blinding. Thirty minutes of computerized arithmetic drill and strategy training was applied. From Tuesday to Thursday, participants only engaged in the stimulation paired with the training. On Friday, after the last session of training, participants received the testing version of the arithmetic problems. Later on the same day, participants went back to perform another scan involving the same sequence of events, including repeated safety screening. Participants were compensated for their time and effort after finishing the last session. After the study was finished, participants were re-invited for a follow-up assessment, in which the testing session was repeated. Here, participants performed the blocks for drill and calculation testing they had performed in their last testing session. No brain stimulation was applied.

6.3 Results

Statistical data were analyzed using IBM® SPSS® 20. In order to control for initial differences in task performance, the first block of the first training session was included as a covariate⁹. Outliers exceeding the mean by 2

⁹ Note that in Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation, the three levels of the within-subjects factor stimulation condition did not allow for the inclusion of the first task block as a covariate, and therefore mathematical reasoning was used as a covariate instead. In the current study, stimulation condition served

standard deviations on the task were removed. The cut-off for significance was set at an alpha of 5% for bidirectional tests and in cases where the assumption of sphericity was violated, Greenhouse-Geisser (GG) correction was performed. Visualizations of the effects contain the mean (average) and standard error of the mean (SEM).

6.3.1 Power

Using G-Power 3.0, I computed the sample size required for optimal power (95%) in the current statistical analyses. The minimum effect size was set at .25 ($\eta_p^2 = .06$) and a correlation between repeated measures assumed to be $r = .5$. For the between-subject effect of stimulation condition of neurochemicals (pre- and post-measures), a total sample size of $N = 189$ and within-subject factor change would require $N = 54$. The interaction would require an N of 66. For the training effects across the five training days, a total sample of $N = 39$ would be sufficient for the within-between group interaction and $N = 33$ for the within-group main effect. The between-group main effect would require an N of 189. An $N = 37$ would be required for Pearson's correlation analyses.

6.3.2 MRS at baseline

Due to the exclusion of MRS data based on quality assurance, the group sizes per scanning session were partly reduced (see Table 6.0.2.1). Measurements with CRLBs > 20% were excluded. This affected mostly GABA. Line widths were below 6Hz.

as a between-subjects covariate and therefore I was able to correct for the initial performance on the main experimental task as in the original study by Snowball et al. (2013).

	Frontal tRNS	Parietal tRNS	Sham	
			<i>Frontal</i>	<i>Parietal</i>
V1				
GABA	23/23	23/22	12/12	12/11
Glutamate	23/23	23/22	12/12	12/11
GABA change	23	21	12	11
Glutamate change	23	21	12	11
VOI tissue contents	24/23	24/24	11/12	12/12
Stimulated VOI				
GABA	17/16	22/20	10/11	12/12
Glutamate	22/22	23/20	11/12	12/12
GABA change	12	18	9	12
Glutamate change	22	19	11	12
VOI tissue contents	24/23	24/24	11/12	12/12

Table 6.0.2.1

Number of voxel measures available per stimulation group. The first number indicates N at pre-scan, and the second number (after the slash) at post-scan. Some voxels had to be excluded entirely, while some provided good glutamate measures and insufficient quality for GABA. Data exclusion at one time-point (e.g. participant 1 pre-tRNS) also eliminated the respective other time point (e.g., participant 1 post-tRNS), therefore the number of data points for change in GABA was further reduced in some cases.

6.3.2.1 Experimental VOIs and Mathematics at baseline

Mathematical abilities were unrelated to glutamate/GABA ($p>.11$), but correlated positively with IPS glutamate and GABA (Table 6.0.3). There were no significant correlations in the MFG or V1.

	Numerical operations	Mathematics reasoning	Composite score
IPS			
Glu/GABA	-.06	.21	.11

GABA	.04	.35*	.24
Glu	.25	.38*	.36*
Creatine	.02	-.2	-.11
MFG			
Glu/GABA	.05	.09	.03
GABA	-.07	.19	.06
Glu	-.19	.004	-.11
Creatine	.2	.1	.18
V1 (N=70)			
Glu/GABA	.07	.1	.08
GABA	.01	.18	.11
Glu	.002	.009	.02
Creatine	-.04	.06	.03

Table 6.0.3

Spearman's correlation coefficients between voxel metabolite and baseline standardized mathematical abilities. Higher IPS glutamate and GABA were associated with superior mathematical abilities. The correlations were not driven by total creatine (* $p < .05$).

6.3.3 Complex arithmetic training

In order to spare participant data when removing outliers (see Table 6.0.4), I performed separate 5x3 repeated measures ANCOVA with stimulation as the between-subject factor and task (drill/calculation) and session (1-5) as within-participants factors for calculation and drill. In order to correct for individual differences at baseline, initial performance (on the first block of each task on day 1) served as a covariate.

Frontal	Parietal	Sham
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Drill	RT	2		
	ACC	4	6	4
Calculation	RT	3		2
	ACC	2	1	3

Table 6.0.4

Number of outliers per condition (cut-off ± 2 standard deviations of the mean).

For calculation accuracy, there was a significant interaction between session and stimulation ($F(6.65,248)=2.1$, $p=.049$, $\eta_p^2=.06$). The effect was due to a significant effect of stimulation on day 2 ($F(2,62)=3.66$, $p=.03$), where the frontal group performed significantly better than parietal ($p=.01$), as well as on the last day ($F(2,62)=7.35$, $p=.001$). On the last day, frontal performed superior to parietal ($p<.001$) and parietal significantly worse than sham ($p=.01$). Drill accuracy showed no significant main or interaction effect with stimulation and day ($p=.33$ and $p=.86$, respectively). Response times of both drill and calculation problems showed no significant main or interaction effects ($ps>.13$).

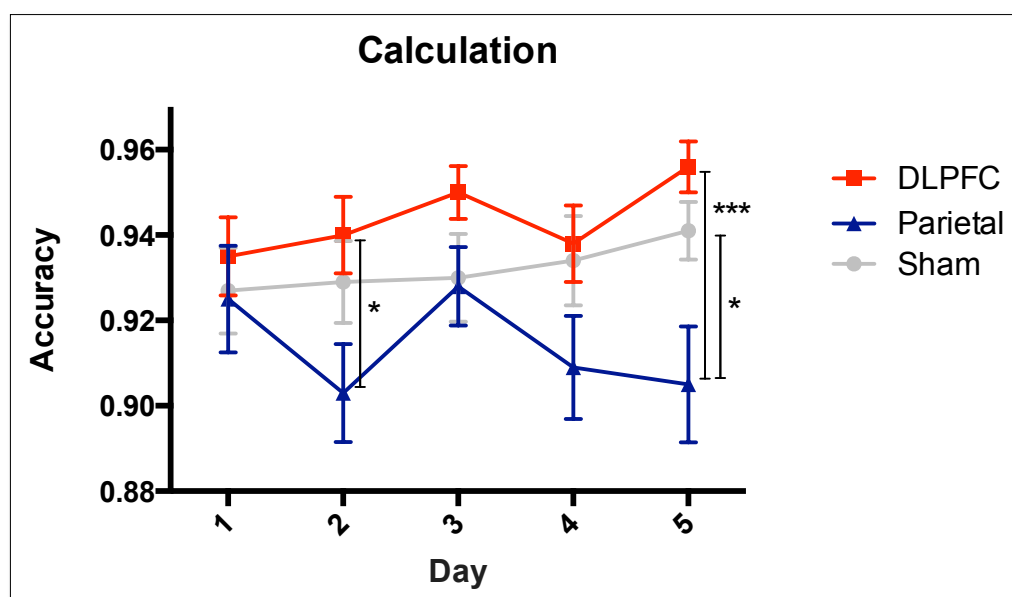


Figure 6.1

Interaction between day and stimulation on calculation accuracy. Frontal tRNS outperformed parietal tRNS on days 2 and 5. On day 5, there was even a significant impairment in the parietal compared to the sham condition (* $p < .05$; *** $p < .001$).

6.3.4 Testing and transfer

Due to the limited availability of data for untrained drill problems, I investigated the effects of testing and transfer for each task separately. Therefore, I performed 2x3 factorial repeated measures ANOVAs, with task block (trained vs. untrained) as a within-subjects factor and stimulation condition as a between-subjects factor. For drill, there was a strong main effect of task block on accuracy, whereby accuracy was higher on the trained than untrained problems ($F(1,64)=610.73$, $p < .001$, $\eta_p^2=.91$). Untrained problems approached a floor effect, with significantly lower accuracy compared to trained problems (Figure 6.3). The effect of stimulation on trained drill response times was not significant ($F(2,64)=.54$, $p=.59$, $\eta_p^2=.02$).

For calculation accuracy, there was a main effect for block type ($F(1,62)=29.27$, $p < .001$, $\eta_p^2=.32$) and a trend for a main effect of stimulation ($F(2,62)=3.03$, $p=.06$, $\eta_p^2=.09$). Accuracy on untrained problems was significantly lower than on trained problems ($F(1,64)=29.42$, $p < .001$, $\eta_p^2=.32$). The trend for a stimulation effect was due to significantly lower accuracy in the parietal group compared to frontal, as well as compared to sham (both $ps=.04$; Figure 6.2).

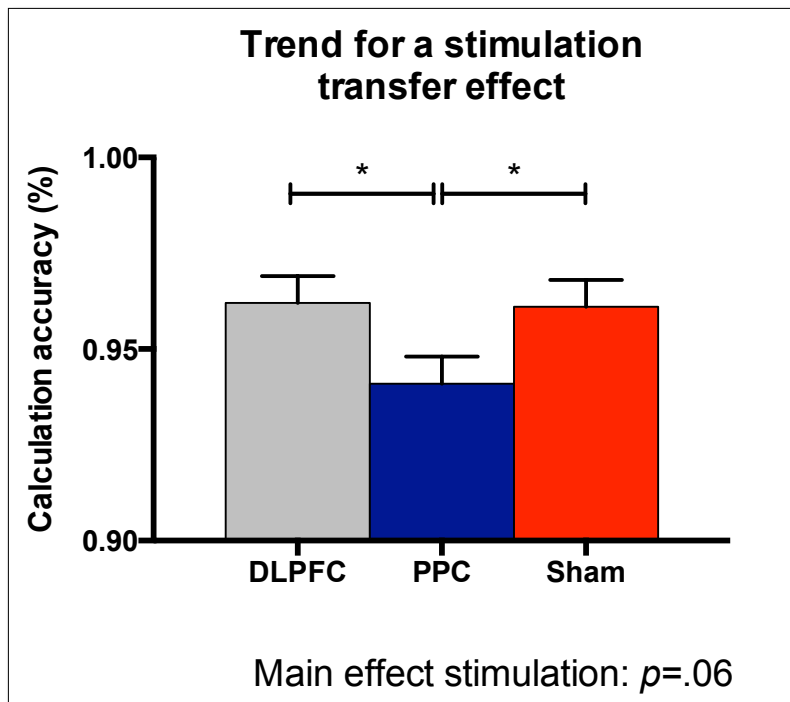


Figure 6.2

Trend for a stimulation main effect on calculation transfer (accuracy in percent). The trend was due to reduced accuracy in the PPC compared to the DLPFC tRNS and sham groups ($*p<.05$).

6.3.5 Follow-up arithmetic testing

For the follow-up testing (ranging between 75 and 449 days post-testing) 15, 17 and 12 participants had returned of the original frontal, parietal and sham stimulation conditions, respectively. I tested whether the inter-testing-follow-up interval was significantly different across stimulation conditions. The effect was marginal ($F(2,41)=3.2$, $p=.051$, $\eta_p^2=.14$). This was due to shorter intervals in the sham than the DLPFC ($p=.03$) and PPC ($p=.03$) conditions (intervals in days: sham mean=128.08, SD=45.95; DLPFC mean=213.4, SD=105.78; PPC mean=213.88, SD=119.82). Accuracy on untrained drill problems was significantly reduced compared to trained problems ($F(1,44)=21.2$, $p<.001$, $\eta_p^2=.33$; close to 14% difference). For calculation, neither main effects nor interaction effects were significant ($ps>.36$). For calculation and drill response times, there were no main or interaction effects with stimulation ($ps>.09$). The results remained similar when I added the interval between testing and follow-up as a covariate ($ps>.08$).

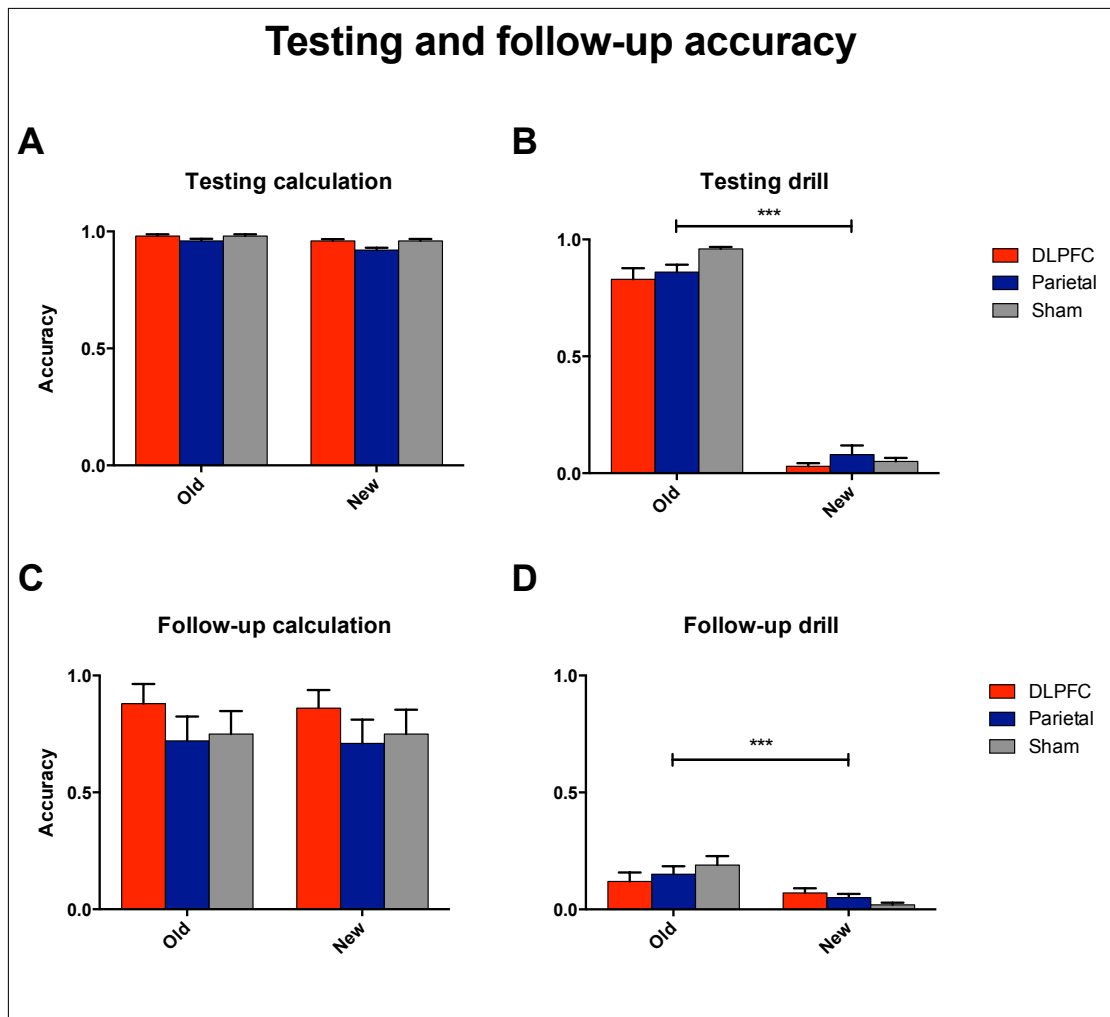


Figure 6.3

Testing and follow-up accuracy. A) Calculation performance transferred well from trained to untrained material in all conditions for calculation. B) As expected, memorizing solutions to problems cannot transfer to untrained problems. Accuracy on new problems (untrained) was close to zero. C) At follow-up, frontal tRNS was not significantly faster than sham or parietal tRNS ($p=.08$), but accuracy was generally high. D) There was little retention of the learned material (old) for drill, and again, close to zero for untrained problems.

6.3.6 MRS results

I performed an initial 2x2x2 factorial repeated measures ANOVAs (area: (V1 vs. stimulated area) x VOI (PPC vs. DLPFC) x time (pre-, post-training) x stimulation (real vs. sham)) for each metabolite. Due to the difficulty to use covariates for two 2-level within-subject factors (area and time), I also

performed 2x2x2 repeated measures analyses of covariance (ANCOVA: VOI x time x stimulation) with the percentage of change in creatine as a covariate (see the next paragraph). Percentages of metabolite changes were calculated: $[(\text{met}_{\text{post}} - \text{met}_{\text{pre}}) / \text{met}_{\text{pre}}] * 100$.

6.3.6.1 Creatine

In the initial 2x2x2x2 interaction, there was a trend for a time x stimulation interaction ($F(1,60)=3.32$, $p=.07$, $\eta_p^2=.05$). There was no stimulation effect at pre ($p=.47$) or post ($p=.14$). There was higher creatine at baseline compared to post-training in the sham group ($F(1,18)=5.93$, $p=.03$, $\eta_p^2=.25$) and this was not the case for tRNS ($p=.49$). The percentage change, however, was not significantly different between groups ($p=.22$). Creatine decreased by ~14% in the sham group and tRNS by less than 1% (mean tRNS=-13.68, SEM=8.91; mean sham=-.56, SEM=5.79). For the following analyses on glutamate and GABA, I added creatine change as covariates, since both were scaled to total creatine. Since the referencing canceled out when dividing glutamate by GABA (glutamate/GABA), this was not necessary for the analysis of glutamate/GABA ratios.

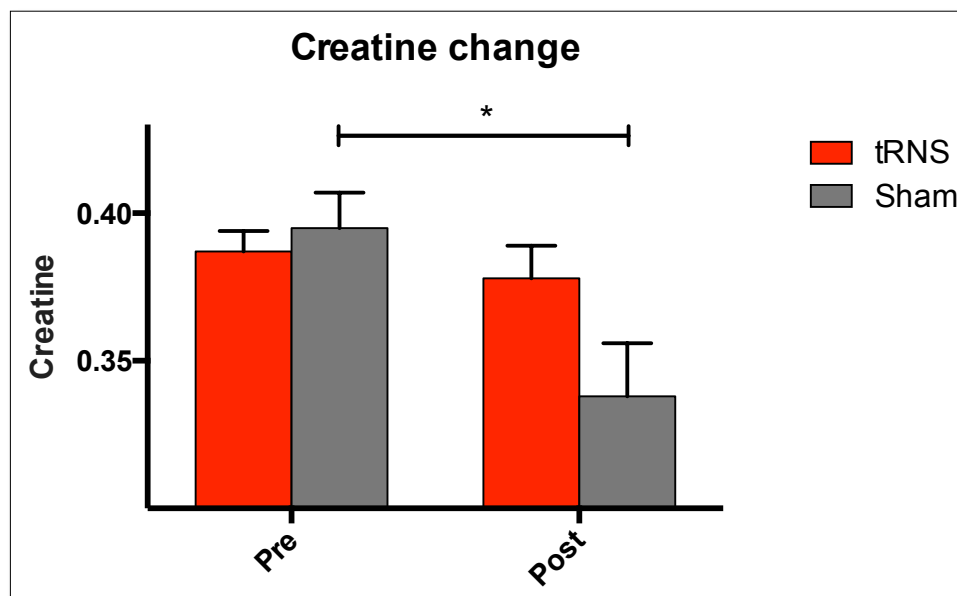


Figure 6.4

Interaction between time and stimulation. Creatine decreased across VOIs from pre- to post-training in the sham, but not the tRNS group ($p<.05$).

6.3.6.2 GABA

There was no main or interaction effect with stimulation (tRNS vs. sham; $p > .21$). There was an area $\times$ time ($F(1,45)=15.49$, $p < .001$, $\eta_p^2=.26$) and an area $\times$ VOI interaction ($F(1,45)=9.66$, $p=.003$, $\eta_p^2=.17$). Further, in order to be able to use creatine change as a covariate, I performed a 2x2x2 repeated measures analysis of covariance (ANCOVA: VOI $\times$ time $\times$ stimulation) with the percentage change in creatine as a covariate. There was a main effect of VOI ($F(1,42)=16.01$, $p < .001$, $\eta_p^2=.28$), demonstrating higher GABA in the IPS (mean=.07, SEM=.004) than the MFG (mean=.043, SEM=.005), independent of the stimulation condition and time. Furthermore, there was a main effect of time ($F(1,42)=7.58$, $p=.009$, $\eta_p^2=.15$), demonstrating a decrease in GABA from pre- to post-training (pre mean=.06, SEM=.004; post mean=.053, SEM=.003). There were no effects in V1 ($ps > .11$).

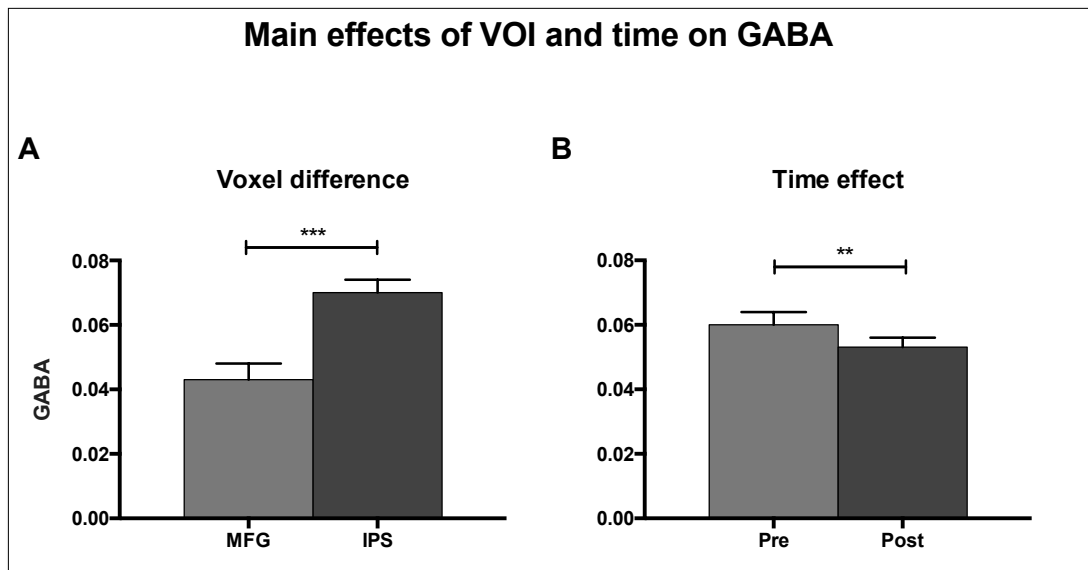


Figure 6.5

Main effects of VOI and time on GABA. A) The IPS had significantly higher GABA than the MFG. B) There was a general decrease in GABA from pre- to post-training (** $p < .01$, *** $p < .001$).

6.3.6.3 Glutamate

There were no main or interaction effects for glutamate ($ps > .21$). In a 2x2x2 repeated measures analysis of covariance (ANCOVA: VOI $\times$ time $\times$

stimulation) with the percentage change in creatine as a covariate, there was a significant main effect of stimulation in V1 ($F(1,60)=4.05$, $p=.049$, $\eta_p^2=.06$). The sham group had overall higher glutamate compared to tRNS (sham mean=1.14, SEM=.022; tRNS mean=1.09, SEM=.014). There were no effects in the VOIs ($ps>.15$).

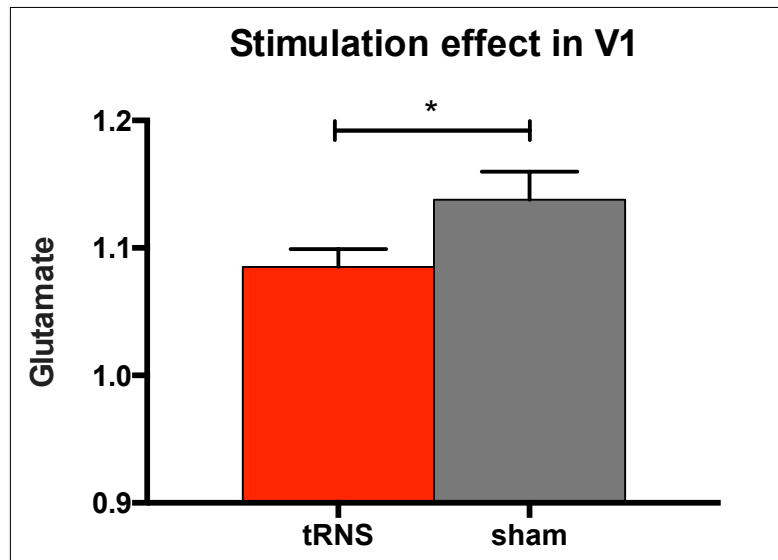


Figure 6.6

Stimulation effect on glutamate in V1. There was a general difference between conditions (* $p<.05$).

6.3.6.4 Glutamate/GABA

There were no main or interaction effects for glutamate ($ps>.15$). Note that creatine was not used as a covariate here because the effect of creatine cancels out by referencing glutamate and GABA to one another.

6.3.6.5 Linking behavioral effects to neurochemicals

The behavioral results showed that in certain cases, PPC and DLPFC differed from each other, but not from sham. The problem with the neurochemical data was the fact that the sham group consisted of two subgroups (frontal and parietal sham). Therefore, in order to be able to link the neurochemical results better to the behavioral results, I also performed an analysis of metabolite changes between the two tRNS conditions only. None of the metabolites changed differentially between PPC and DLPFC tRNS ($ps>.33$).

6.3.7 Left vs. right IPS and Mathematics

In order to investigate the relationship between basic mathematical abilities in the left and right IPS, I here present Spearman's correlations from female adult participants (right IPS, N=14; from the experiment in chapter 'Inhibition, excitation, and cognitive achievement in the developing brain') and from the female sub-group of this sample (left IPS, N=18). There were no neurochemical-mathematics correlations in the right IPS ($ps > .31$). In the left IPS, glutamate/GABA ratios correlated with mathematical reasoning ($r_p = .56$, $p = .02$; other $ps > .07$). Glutamate was correlated with all standardized sub-scales, and GABA with mathematical reasoning and the composite score. However, only the correlation between mathematical reasoning and glutamate/GABA was significantly different between hemispheres (Fisher's $Z = 12.21$, $p < .05$).

	Numerical operations	Mathematical reasoning	Composite score
Left IPS			
Glutamate/GABA	-.18	-.56*	-.45 ^{trend}
Glutamate	.52*	.48*	.57*
GABA	.29	.53*	.5*
Right IPS			
Glutamate/GABA	<.001	.24	.13
Glutamate	.24	.29	.29
GABA	.19	-.02	.1
<i>Fisher's Z test to compare groups</i>			
Glutamate/GABA	-0.46	-2.21*	-1.55
Glutamate	0.84	0.57	0.88
GABA	0.27	1.54	1.13

Table 6.0.5

Spearman's correlation coefficients in women from two studies reported in this thesis (* $p < .05$).

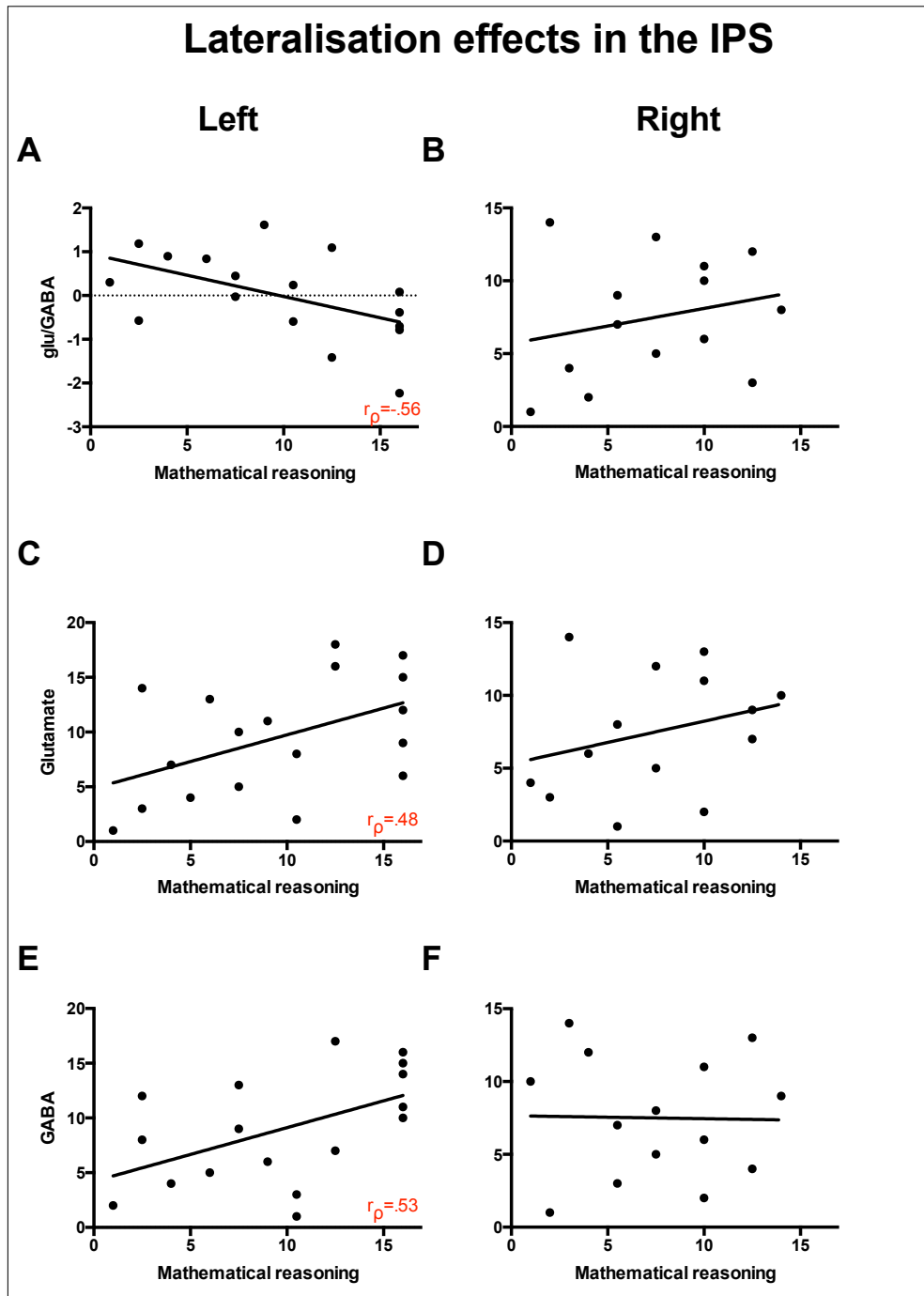


Figure 6.7

Spearman's non-parametric correlations between mathematical abilities and neurochemicals were only significant in the left, not the right IPS. The x-axis shows ranked standardized scores for mathematical reasoning and the y-axis the concentration of neurochemicals in the respective area. A) Higher glutamate/GABA ratios were associated poorer mathematical abilities in the left, but not the right (B) IPS. C) Higher levels of glutamate and E) GABA in the left, but not the right (D,F) IPS were associated with superior mathematical abilities.

6.4 Discussion

At the behavioral level, tRNS to bilateral PPCs led to worse calculation accuracy on the fifth day compared to both sham and frontal tRNS. PPC tRNS also led to worse calculation accuracy than frontal tRNS also on the second day and there was a trend for reduced performance in the testing session, which assessed accuracy across trained and untrained calculation problems. The difference on the second day was ca. 4%, and remained 5% on the fifth day. There were no further behavioral effects. Importantly, the PPC tRNS showed no evidence of impairments at long-term follow-up. At the neurochemical level, there was a decrease in creatine from pre- to post-training across all voxels (V1, MFG and IPS) in the sham group, but not the tRNS group. The percentage of change did not, however, differ between conditions. GABA was higher in the IPS than the MFG and decreased across voxels from pre- to post-training. In addition, I found no effects for glutamate in the stimulated VOIs, but in V1, the sham group had higher levels of glutamate compared to tRNS, but this effect did not interact with time. For glutamate/GABA ratios, there were no effects. The more participants improved on drill-based training, the larger the increase in GABA.

This study aimed to test whether bilateral prefrontal and parietal tRNS can improve arithmetic training effects and simultaneously modulate regional concentrations of GABA and glutamate. Surprisingly, the improved performance under tRNS to bilateral DLPFCs (Snowball, et al., 2013; and 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation') could not be found here. DLPFC

tRNS only performed better than PPC tRNS during training and testing, but not compared to sham. However, tRNS to bilateral PPCs impaired calculation performance. This effect mainly occurred on the fifth day of stimulation but also affected calculation accuracy in the testing session, in which trained and untrained problems were presented. It is therefore possible that the accumulated effect of the stimulation is disadvantageous for such complex arithmetic training. It is important to note that this impairment involved a ca. 5% reduction in accuracy compared to sham, which may impact real-life arithmetic functioning. However, this impairment in performance of the PPC tRNS group was no longer visible at follow-up.

Despite the initial impairment under PPC tRNS, there were no significant differences in neurochemicals due to stimulation. I observed a decrease in creatine across all voxels across the training period, under sham, but not tRNS. Besides a generally higher concentration of glutamate in the sham group than the tRNS group, there were no further stimulation effects on neurochemical concentrations. Previous learning studies have demonstrated that behavioral improvements were associated with changes in neurochemicals (Floyer-Lea, et al., 2006; Sampaio-Baptista, et al., 2015). It is therefore not surprising that I was unable to find changes in glutamate and GABA under DLPFC tRNS. Since the impairments under PPC tRNS were limited to certain sub-parts of the training and testing paradigm, it is possible that this impairment was too specific and subtle to be reflected in neurochemical changes measured in a 2x2x2cm volume. It is also important to know that while I was able to track behavioral performance across five days of training and testing, neurochemical measures were only available before and after the training period. It is therefore unclear whether neurochemicals have changed throughout the period of training.

It is unclear why the current experiment failed to replicate behavioral results from previous studies (Snowball, et al., 2013; 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation') but it is possible that sample-specific effects have led to the differences in outcomes. Impairments with PPC stimulation on specific aspects of numerical or arithmetic abilities, while improvements in other aspects, have been found by several groups (Cohen Kadosh, Soskic, et al.,

2010; Iuculano & Cohen Kadosh, 2013; Rutsche, et al., 2015). Others found no impairments but only improvements (Cappelletti, et al., 2013; Hauser, et al., 2013; Snowball, et al., 2013). It is possible that in this study, inter-individual differences in brain functioning, anatomy, and cognitive state have affected the effects of tRNS on both behavioral and neurochemical levels (Krause & Cohen Kadosh, 2014). Furthermore, it is possible that the choice of the voxels (left prefrontal and parietal) has led to a masking of a potential stimulation effect on neurochemicals. Unfortunately, it was not possible to assess both hemispheres for each voxel, but I cannot exclude the possibility that effects looked different in the right DLPFC and IPS.

In addition to the effects of tRNS, I used the female sub-sample to compare neurochemical levels the left IPS (current study) and the right IPS from the adult sample from the chapter 'Inhibition, excitation, and cognitive achievement in the developing brain' in relationship with standardized mathematical abilities. The strategies for the manual placement of the voxels was the same across the two studies but involved different hemispheres. While I was unable to find behavior-neurochemical relationships in the right IPS, glutamate, and GABA, were positively, and glutamate/GABA ratios negatively associated mathematics in the left hemisphere. Accordingly, superior mathematical abilities were associated with higher glutamate and GABA, but reduced relative glutamate-to-GABA concentrations in the left IPS. Functional lateralization effects have been found for different types of quantity, arithmetic, and learning processes (Cohen Kadosh, Muggleton, Silvanto, & Walsh, 2010; Cohen Kadosh, Soskic, et al., 2010; Rosenberg-Lee, et al., 2011; Zamarian, et al., 2009). Due to the complexity and particular sensitivity of MRS to noise and other factors, the current study had to face several weaknesses, which were difficult to avoid at such a large-scale experiment. First, due to limited possibilities for scan times, only two VOIs could be assessed per participant. This resulted in a sham group of 24 participants, who had to be split into 12 MFG and 12 IPS VOIs. This has led to difficulties in comparing the effects between the groups, as the separate sham groups were now half the size of the two experimental ones. A related problem is that I stimulated bilateral frontal or parietal areas, but was only able to investigate neurochemical changes in one VOI under the electrode. In

addition to the small sham groups, it would also have been interesting to investigate whether stimulation to one region (e.g. PPCs) had an effect on neurochemicals in the frontal cortex. Brain imaging studies have demonstrated that the effect of the stimulation to one site can affect other parts of the network, and is not limited to the area under the electrode (Keeser, et al., 2011; Zheng, et al., 2011).

Second, some of the measurements for GABA, especially in the MFG, had to be excluded due to reduced data quality. This demonstrated the difficulty in assessing GABA in the frontal cortex, where MRI signal dropout is generally higher compared to more posterior areas. Third, I have previously discussed the variety of factors that can influence the effect of tES, such as age, gender, pre-existing concentrations of GABA and glutamate, mental state, and experimental conditions (Krause & Cohen Kadosh, 2014). Since I matched the stimulation groups to balance age, gender, and basic mathematical abilities (WIAT-II scores), I controlled for some of the most important factors. Unfortunately, the group sizes (especially after removal of insufficiently qualified data) were not large enough to perform more detailed analyses on individual contributions or the prediction of behavioral changes based on baseline concentrations. Even though the same training paradigm was used repeatedly before (Snowball, et al., 2013; 'Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation'), the effects of tRNS on this type of arithmetic training did not replicate previous findings. An exact explanation for this phenomenon is not currently available.

6.5 Conclusion

The current results suggest that tRNS to bilateral PPCs during five days of arithmetic training can impair learning and subsequent performance on trained and novel material. The impairments, however, were not found at follow-up testing months after the training period. I recommend great caution when using such stimulation aimed to affect people's cognitive abilities, and future studies should investigate the link between neurochemical and cognitive changes in greater detail and with enhanced experimental power.

The study also emphasizes the important role of replication studies for the understanding of noninvasive brain stimulation effects. I will provide a more detailed overview of the different outcomes using this study paradigm in the chapter 'General discussion'. Even though one study may provide strong effects, it cannot be assumed that the effects can be replicated 1-by-1 in another study involving different participants.

7 General discussion

In this thesis, I aimed to answer five main research questions:

1. Can previous results from our lab on the effect of tRNS on arithmetic training be replicated using a different study design?
2. Are glutamate and GABA in brain areas involved in mathematical abilities associated with cognitive measures of mathematics? Are they only associated separately or is the ratio of glutamate and GABA associated with mathematics? Are potential associations different in children and adults due to developmental changes in the brain?
3. Are glutamate and GABA concentrations in brain areas associated with mathematics distinct in an individual with exceptional calculation abilities?
4. Is it possible to boost calculation performance of an individual with already exceptional abilities using excitatory tES? Can the performance of expert calculators who are not prodigies be further improved by excitatory tES?
5. Can glutamate and GABA concentrations be modulated using excitatory tES during arithmetic training?

Here I will discuss these questions in the light of my experimental results.

7.1 Summary of findings

In the experiments presented in this thesis, I investigated how regional, noninvasive measures of glutamate and GABA in a fronto-parietal brain network were related to numerical and arithmetic abilities. My target populations were largely individuals with average to above-average mathematical abilities. In addition, I compared neurochemical-mathematics relationships within this network in a child sample and a calculation prodigy or 'lightning calculator'. Given that MRS research has demonstrated the relationship between glutamate, GABA and their modulation by learning and tDCS (Clark, et al., 2011; Floyer-Lea, et al., 2006; Sampaio-Baptista, et al., 2015; Stagg, et al., 2011; Stagg, et al., 2009), I combined MRS measures with

a newer method of tES, namely tRNS, to investigate whether tRNS can modulate neurochemicals and cognitive training.

7.1.1 TES in arithmetic training

I began to answer my research questions by examining whether previous results demonstrating the effect of tRNS on arithmetic training, could be replicated using a different study design ('Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation'). The initial study (Snowball et al., 2013) found improvements in drill and calculation learning under tRNS to bilateral DLPFCs but not PPCs. I found that response times for calculation, but not drill problems, were faster under tRNS than sham stimulation. I replicated the study in a within-subject instead of a between-subjects design. Contrary to the previous results, I found improvements in calculation, but not drill speed, under tRNS to bilateral PPCs, but not DLPFCs. This was despite an initial advantage in calculation speed in the sham condition at baseline. Additionally, calculation accuracy was also improved in the PPC tRNS condition, while DLPFC tRNS did not differ from sham. Surprisingly, the DLPFC tRNS group then showed improved transfer from the trained to untrained calculation material: they performed faster on novel problems than sham and PPC tRNS. However, they did not differ on the trained problems. This study is relevant to the field of cognitive tES research for the following reasons: using the same training paradigm and stimulation parameters, the effects cannot always be replicated. In my study, the influence of certain individual differences was reduced due to the within-participant design. The wash-out period of two weeks in between tRNS-training periods was intended to eliminate carry-over effects. A critical feature of the design by Snowball et al. (2013) was the persistence of the positive effect after six months post-testing. I therefore cannot exclude the possibility that there were residual effects of the stimulation on the next training period. My within-subject design did not allow to test such long-term effects in a comparable way. However, I counter-balanced the order of stimulation across the sample to minimize this effect as much as possible. Furthermore, I controlled for participants' general mathematical abilities. Due to the design with three within-subject levels of the factor of stimulation condition, I could

not validly use the performance on the first block of the task as a covariate as was done in the study by Snowball et al. (2013). It is possible that this difference has contributed to additional variations in results. The sample size is a further limitation, as a small repeated-measures sample may be more prone to subject-related or sample-specific effects. I have previously discussed the possible influence of individual differences on the effects of tES on behavior and neurophysiology (Krause & Cohen Kadosh, 2014). This topic has also recently been picked up by other scientists and is now a subject of discussion in the field (Li, Uehara, & Hanakawa, 2015; Ridding & Ziemann, 2010; Wiethoff, Hamada, & Rothwell, 2014). It is therefore important for researchers and clinicians considering tES as a possible treatment to be aware that a single study at a group comparison level does not provide sufficient information to apply the same stimulation strategy to every individual.

This raised a different question, namely: can performance be enhanced using tES in an individual with exceptional cognitive abilities? I stimulated G.M.'s (a calculator with extraordinary abilities outside the range of standardized scales) bilateral DLPFCs using tRNS and sham and found that across six sessions, there were no improvements in his extraordinary calculation performance ('Neuroenhancement of High-Level Cognition: Pushing the Boundaries of the Human Brain'). In order to support my findings, I tested an additional six individuals who were postgraduates in mathematics- and statistics-related fields at the university. Using the same stimulation parameters, I found impaired performance under tRNS compared to sham in this within-subjects design. Without doubt, such small-scale studies require careful interpretation, but from this sample, I concluded that it is indeed possible that highly proficient individuals can be impaired under the use of tES. This study further corroborated my hypothesis that individual factors are likely to determine the outcome of tES (Krause & Cohen Kadosh, 2014). This view also suggests that the usage of tES for clinical applications might be premature at this point. First, we need to gain a better understanding of the underlying factors leading to improvements or impairments, and to be able to predict the stimulation effects in different individuals and populations. It is important to avoid potential cognitive impairments and therefore potential risks

need to be weighed against benefits and regulations may be required (see Cohen Kadosh, et al., 2013; Cohen Kadosh, et al., 2012; Maslen, et al., 2014; Maslen, et al., 2013).

In the chapter 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation', I replicated the same paradigm as a between-subjects study once more at a large scale (N=72) in order to investigate neurochemical changes under tRNS (see 'The modulation of arithmetic learning and glutamate and GABA'). In this study, the behavioral results differed from the within-subjects study in the chapter Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation, but also from the initial study by Snowball et al (2013). There were no significant improvements under tRNS to bilateral DLPFCs compared to sham, but impairments under tRNS to bilateral PPCs. These impairments occurred during the training but also for some of the transfer tasks. However, at follow-up several months post stimulation, there was no longer any evidence of impairments. Below a summary of results of all three available studies using the arithmetic training-tRNS paradigm in (Table 7.1).

Study	tRNS to DLPFCs	tRNS to PPCs
(Snowball, et al., 2013)	Improved drill and calculation learning (ACC). Faster calculation RTs (median) on trained and untrained problems. Six months post-testing tRNS was still better for calculation problems but not drill.	No effects
Modulating prefrontal and parietal cortices	Faster calculation RTs on untrained material	Improvements in calculation RTs across

to enhance arithmetic learning using transcranial random noise stimulation	(transfer effect).	training days despite slow initial performance, and overall ACC improvement.
Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation	No effects.	Calculation accuracy impaired on the fifth day and poorer than DLPFC tRNS on day 2. There was a trend for impaired calculation accuracy on trained and untrained problems at testing. No impairment found at long-term follow-up.

Table 7.1

Summary of results from replications of the original Snowball et al. study (Snowball et al., 2013). The differences suggest sample-specific variations in response to tRNS.

7.1.2 The role of the stimulated areas

The electrode montage is decisive for the outcome of stimulation interventions. The mathematical network is very complex and widespread and with the range of choices come differences in cognitive effects. For example, a widely discussed topic in the literature is whether certain types of calculation rely on unilateral or bilateral processing. This is important as noninvasive brain stimulation not only affects the stimulated brain areas, but also modulates interhemispheric inhibition and patterns of connectivity between bilateral brain areas (Blankenburg et al., 2008; Heinen et al., 2011; Park et al., 2013; Vines, Cerruti, & Schlaug, 2008). This means that the enhancement of one brain area exerts a certain influence on the same area in the contralateral hemisphere. TRNS is considered to increase cortical excitability, and

therefore to facilitate neural processing in the stimulated area(s) (Terney, et al., 2008). However, the cognitive functions associated with left and right hemispheres in the prefrontal and parietal cortices for arithmetic are not entirely symmetrical and it is unclear how interhemispheric inhibition will affect cognitive outcomes when bilateral brain areas are stimulated. Since the studies presented in this thesis work only involved bilateral stimulation, the effects of the stimulation on each individual hemisphere cannot be disentangled. It is possible that the enhancement effects on one hemisphere were masked by the simultaneous stimulation of the other hemisphere. For the exact understanding of laterality effects in such designs, a unilateral stimulation condition would be crucial. In the following, I will outline some of the functional evidence on laterality in mental arithmetic. This discussion demonstrates the complexity of the brain mathematical network and the challenge to predict the effects of (bilateral) tES on those areas.

7.1.2.1 Hemispheric effects

The complex arithmetic training task used in the chapters ‘Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation’ and ‘Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation’ targeted a range of different cognitive sub-components, each likely to exhibit a certain degree of hemispheric differences. The strategy problems in the current five-day training paradigm consisted of combinations of either multiplication and addition, or multiplication and subtraction problems. Furthermore, drill problems were entirely based on numerical fact retrieval. Generally, addition, subtraction and multiplication processing is mainly found in frontal/prefrontal and parietal areas, which would be accessible for electrodes placed over F3-F4 and P3-P4 (Arsalidou & Taylor, 2011). Arithmetic facts retrieval has been consistently associated with the AG, but to a certain degree and under certain conditions also with the IPS (Andres, Pelgrims, Michaux, Olivier, & Pesenti, 2011; Grabner et al., 2009).

Both DLPFC and PPC are fundamental parts of the mathematical brain network but have been associated with a multitude of cognitive tasks, which often complicates the interpretation of fMRI results (Arsalidou & Taylor, 2011;

Benn, Zheng, Wilkinson, Siegal, & Varley, 2012; Dehaene, Molko, Cohen, & Wilson, 2004; Houde, et al., 2010; Kaufmann, et al., 2011). Multiplication is more related to the rote memorization of facts, whereas subtraction is thought to require more actual computation, engaging parts of the IPS (Rosenberg-Lee, Lovett, & Anderson, 2009). In a large meta-analysis, left MFG activation was found for all three types of calculation with a certain degree of overlap and a relatively bilateral distribution (Arsalidou & Taylor, 2011). However, smaller differences could be identified. For example, right MFG activation was less pronounced for addition. Similarly, left PPC showed clusters for all types of problems, whereas the right PPC was relatively limited to subtraction and small areas of addition, and only little was dedicated to multiplication. The authors also found that calculation was associated more with prefrontal areas when compared to numerical tasks, which they attributed to the increased task working memory load.

This large meta-analysis provides a broad picture of arithmetic functioning. However, individual studies differ slightly in their results regarding laterality effects. For instance, it has been found that PPC areas were not activated, but even deactivated during addition (Rosenberg-Lee, et al., 2011). Furthermore, the same study found left but not right IPS and superior parietal lobe (SPL) activation and some right AG activation for subtraction, again suggesting laterality effects. Lastly, bilateral PPC activation was found for multiplication. Deactivations were also found here for right middle-to-superior prefrontal cortices during addition and subtraction. TES effects may interact with cognitive functions of opposing activation patterns (activation vs. deactivation), which adds to the complexity of the intervention. In another study, subtraction was found to be represented more strongly in the left than the right posterior brain areas, and the clusters here were larger for subtraction than for addition (Benn, et al., 2012). On the contrary, in the frontal cortex, addition appeared to be more prominent in the left than the right hemisphere. Such differences in findings seem to be due to different task designs and accordingly the engagement of differing brain areas. The exact foci of brain activity in the mathematical network appear to depend on the processing demands, such as problem size, working memory load, verbal or

spatial components of the computations, and to a certain degree also individual solving strategies.

Causal evidence, however, can only be gained from manipulation studies. Evidence from TMS suggests that automatic magnitude processing depends on the right, but not the left IPS (Cohen Kadosh, et al., 2007). Another TMS study has demonstrated that both left and right IPS are crucial for exact calculation (for both addition and multiplication) (Salillas, Semenza, Basso, Vecchi, & Siegal, 2012). However, a closer analysis revealed that the horizontal part (HIPS) was only crucial for multiplication response times in the left, but not the right hemisphere, whereas addition was affected by TMS to either hemisphere. The right ventral HIPS was related with efficiency and response times depended on the difficulty of the problem, as was found in a subsequent regression analysis. In another TMS study, the exact foci of subtraction and multiplication problem processing were established using fMRI and subsequently used as stimulation sites (Andres, et al., 2011). The main areas found here were the HIPS and the posterior superior parietal lobe for both problem types. Left and right HIPS stimulation led to prolonged response times for both problem types and increased multiplication error rates over the HIPS. Due to such fine-grained differences in subparts of the area, tES adds further complexity by its relatively large electrode size. Such fine-grained effects may be better targeted using (unilateral) HD-tDCS.

Given the broad body of evidence, a bilateral electrode design makes sense in the current scientific framework and using the current complex training paradigm. However, due to the lack of effects in some cases, including the expert calculator G.M., it is unclear how the bilateral stimulation has affected the results. For future studies, it would therefore be beneficial to compare bilateral tRNS to unilateral montages to disentangle the laterality effects and investigate the hemispheric interactions in more detail and to prevent the potential masking of effects. Furthermore, for a more detailed understanding and better control of tES on addition, subtraction and multiplication, it would be helpful to use separate tasks for each problem type.

7.1.2.2 Calculation strategies and cognitive demands

A calculation problem can be solved in different ways and despite efforts to experimentally control how participants approach a given task, a certain range of possibilities can be expected. For instance, the number of strategies applied to solve multiplication problems is highly versatile and differs with the existing skill set and the level of expertise, which also affects whether an individual's strategies lead to successful calculation outcomes (Dowker, 1992). Randomizing participants across frontal or parietal stimulation conditions therefore provides no control over which brain areas the individual preferentially engages in a given task. Different strategies can engage different degrees of working memory load, which was directly investigated in a study using fMRI (Rosenberg-Lee, et al., 2009). The authors found that two different strategies engaged the same network of brain areas, but the temporal pattern of activation could be distinguished by the type of strategy and revealed a dissociation: one strategy engaged the left, whereas the other strategy engaged the right hemisphere. The posterior superior parietal lobe (PSPL) consistently distinguished the two strategies and part of the IPS revealed a difference between strategies only when magnitude processing was involved.

Besides individual differences in calculation strategies, training and practice also lead to a shift in processing from frontal to parietal brain areas. Earlier stages of the training rely more on frontal brain areas due to more effortful computations, while processing switches to mainly parietal recruitment once the trained material is processed in a more automatic way and stored information can be more readily retrieved (Delazer et al., 2005; Rivera, et al., 2005). As mentioned above, it is therefore thought that the role of the prefrontal cortex involves the working memory and manipulation of the task material, while the parietal cortices are related to the processing of quantity (IPS) and arithmetic fact retrieval (AG) (Grabner, et al., 2009). Increasing brain activity is thereby observed with increases in task complexity (Menon, Rivera, White, Glover, & Reiss, 2000). The left DLPFC has been associated with verbal, and the right with spatial working memory, whereby task-irrelevant information enhances DLPFC processing of top-down control (Sandrini, Rossini, & Miniussi, 2008). Bilateral IPS are known to be involved in

the processing of quantity, multiplication is one of the functions associated with the AG (Cohen Kadosh, Muggleton, et al., 2010; Dehaene, et al., 2004; Gebuis, Cohen Kadosh, de Haan, & Henik, 2009; Grabner, et al., 2009; Rosenberg-Lee, et al., 2011). In the current arithmetic training task, symbolic quantity processing, multiplication and fact retrieval were crucial parts of the task and the electrodes likely covered parts of both cortical surfaces (see Delazer et al., 2005).

Due to the potential of the stimulation in my training studies to affect the functioning of cognitive domains other than arithmetic, I also assessed verbal and visuo-spatial working memory spans (Kessels, van Zandvoort, Postma, Kappelle, & de Haan, 2000; Wechsler, 1981), as well as spatial-numerical abilities (number-space mapping) before and after the five-day training period ('Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation'; and 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation'). In the latter, I additionally assessed attentional measures using the attention network test (Fan, McCandliss, Sommer, Raz, & Posner, 2002). However, there were no trends or effects (including baseline-training correlations) of brain stimulation on any of the measures, such that the results were not included in the current thesis work.

In summary, individual differences in calculation strategies and therefore brain processing and efficiency may interact, or even remain absent with the effects of the stimulation. Non-arithmetic cognitive functions also associated with the stimulated brain area have the potential to confound, mask or be impaired by the stimulation. The former issue is currently difficult to control, whereas the latter point can be partly controlled by assessing before- and after measures of cognitive functions, such as verbal and visuo-spatial working memory, attention, and simpler numerical abilities. If we want to move to more efficient stimulation strategies, future studies should engage in controlling such factors carefully.

7.1.3 Linking glutamate and GABA with mathematics

My next focus of investigation was to find out whether glutamate and GABA, neurochemicals strongly involved in learning and experience-related neural

plasticity (Ge & Dani, 2005; Trepel & Racine, 2000; Turrigiano & Nelson, 2000), are related to cognitive abilities, when measured in brain areas known to be strongly involved in the cognitive domain in question. This is important for two reasons: 1) linking such neurochemicals to abilities would be beneficial for the (ideally early) identification of poor cognitive abilities or even cognitive impairment; 2) both glutamate, as the major excitatory neurotransmitter in the brain (Ge & Dani, 2005; Trepel & Racine, 2000), and GABA, the hypothesized gate-keeper of the induction of neural plasticity (Hess & Donoghue, 1994), are potential targets for artificial modulation of impaired cognitive ability. Neuromodulation techniques, such as tES aim to re-open what is called the 'sensitive periods' of increased levels of plasticity (Bavelier, Levi, Li, Dan, & Hensch, 2010; Hensch & Bilimoria, 2012). While the child brain is very plastic and adaptive to novel experiences, the adult brain is more stable and it can be difficult to re-shape existing patterns (Knudsen, 2004). The experiment in the chapter 'Inhibition, excitation, and cognitive achievement in the developing brain' was the first step to investigate whether neurochemical-behavior relationships can be demonstrated in normal to above-average calculators and whether such relationships have the potential to change across developmental stages using a cross-sectional design. This hypothesis was based on available evidence of developmental changes in the involvement of the fronto-parietal network in mathematics (Houde, et al., 2010).

From the three MRS studies included in this thesis, I collected glutamate and GABA measures from the left IFG and the right IPS of 15 children and 15 adults (all female, in the chapter 'Inhibition, excitation, and cognitive achievement in the developing brain'), the right DLPFC, right IPS and left V1 from a male expert calculator with prodigious abilities and four age-matched male controls who had superior mathematical abilities who were not prodigies ('The neurochemistry of a genius: substantial alteration in frontal excitation/inhibition balance'). Furthermore, I collected neurochemical data from V1 in 72 participants, as well as 36 of the left DLPFC and 36 of the left IPS (thereof 18 women; 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation'). Without the repeated measures in one study, this equaled 117 participants I acquired MRS data for, and 219 voxels in total. With the addition of the repeated

measures, I acquired 363 voxel scans in total. Even though power was a problem in some cases, the studies combined provided a consistent pattern of results. Even though there was no relationship between glutamate and/or GABA and basic mathematical abilities in the right IPS, I found that higher glutamate and GABA levels in the left IPS were associated with superior individual abilities. There was also a negative correlation for glutamate/GABA, showing that reduced glutamate relative to GABA levels were associated with superior mathematical abilities. These results in the left IPS were specific to the female sub-sample, but there was no glutamate/GABA and mathematics relationship across genders or in males only. The glutamate/GABA relationship was positive in the left IFG of children and an expert calculator with exceptional mathematical abilities showed distinctly low ratios of glutamate/GABA in the right DLPFC compared to four mathematicians and physicists. No neurochemical-mathematics relationships could be found in the left MFG or a control region, V1. I conclude that there are indeed neurochemical-mathematics relationships, which, however, underlie an interaction between brain area and individual features, including level of ability, development, or gender. The mechanism is likely to be more complex than can currently be predicted from fMRI studies on mental arithmetic.

7.1.4 The modulation of arithmetic learning and glutamate and GABA

In the chapter 'Modulating arithmetic abilities and learning-related neurochemicals using transcranial electrical stimulation', I investigated, whether the learning-related neurochemicals glutamate and GABA could be modulated using tRNS. Besides a general decrease in creatine across the training period, I found no changes in glutamate, GABA, or the glutamate/GABA ratio. This was surprising for the following reasons: 1) baseline GABA and glutamate are correlated with performance in various different functional domains (e.g. Boy, et al., 2011; Boy, et al., 2010; Silveri, et al., 2013; Stagg, et al., 2011; Terhune, et al., 2015; Terhune, et al., 2014); 2) learning has been associated with reductions in GABA measured by MRS (Floyer-Lea, et al., 2006; Sampaio-Baptista, et al., 2015); 3) the use of excitatory A-tDCS increases cortical excitability and decreases GABA, whereby the reduction in GABA is correlated with improvements in

performance (Kim, et al., 2014; Stagg, et al., 2011; Stagg, et al., 2009); 4) there is also evidence that glutamate decreases due to A-tDCS, while improving symptom profiles of clinical patients (Foerster, et al., 2015).

Some important and recent work in the field of tES demonstrated that some individuals show responses (enhancement of cortical excitability) to tES, while others do not (Lopez-Alonso, Cheeran, Rio-Rodriguez, & Fernandez-Del-Olmo, 2014). The authors of this study used TMS-based measures of cortical excitability and found that there were excitability increases in approximately 50% of the participants, while the other 50% showed no increases. In cognitive studies, it is difficult to investigate immediate changes in excitability and tES responses directly. Therefore, using MRS to measure glutamate and GABA in combination with tES intervention in cognition is a possible alternative. Unfortunately, the current study lacked the power to investigate the effect of such individual factors¹⁰. Ideally, simpler paradigms, such as motor and perceptual learning, will be used to investigate this topic more thoroughly in the future and provide better insight into the underlying mechanisms of the individual response to tES before moving to more complex cognitive functions. In the light of the stimulation study with the expert calculator G.M., this suggests that there is a possibility that he was one of the non-responders.

7.2 Limitations of the current work

In my studies, I strictly controlled for age, gender, basic mathematical abilities, and sham-controlled blinding and/or counter-balancing of stimulation conditions, training-related stimulus material and screening for safety. Ideally, a control for the menstrual cycle period or hormonal contraception would have been available. Furthermore, there are other factors affecting the effects of tES that are currently unknown. It is also possible that varying the stimulation parameters, such as current type, intensity, electrode size and stimulation duration, would influence the current results. However, I selected the parameters based on literature reporting positive effects of low-intensity tRNS

¹⁰ The relevant study was only published after my tRNS-MRS study was already completed, such that the design could not be adapted to account for such individual variation.

on excitability, cognition, and skin perceptions due to the current (subject-blinding) (Ambrus, et al., 2010; Cappelletti, et al., 2013; Fertonani, et al., 2011; Moliadze, et al., 2012; Snowball, et al., 2013; Terney, et al., 2008). Similar to other forms of tES, however, a certain degree of impairment has also been found in some cases using tRNS (Ambrus et al., 2011).

Since all of my studies involved tRNS and/or MRS, the recruitment was relatively difficult, since my safety criteria for both were strict and therefore excluded numerous potential volunteers. The availability of children in and around Oxford for the chapter 'Inhibition, excitation, and cognitive achievement in the developing brain' was problematic, despite prolonged efforts of recruitment. Therefore, it is unfortunate that the number of participants was low in some cases. The first study ('Modulating prefrontal and parietal cortices to enhance arithmetic learning using transcranial random noise stimulation') only involved 10 participants in total. However, the study was logistically difficult to coordinate, since participants had to perform the same training paradigm three times over five consecutive days each, and an exact gap of two weeks in between two repeated measures periods to keep the design constant. It was therefore difficult to find sufficient numbers of participants who were able to make such a commitment, arrive on time, abstain from alcohol during the periods, and meet the safety criteria. Another problem was that I tested university students with above-average abilities. This may lead to differences in effects compared to average or below average calculators and does not necessarily reflect how the majority of the population responds to the stimulation (or performs on arithmetic tasks). I would like to note that studies should additionally be performed in individuals with average and below average cognitive abilities before the use of tRNS can be considered for potential clinical use.

For the use of both tRNS and MRS in my studies, information about the menstrual cycle would have been useful additional control for participant variation, but was not systematically assessed. I became aware of the discussion of menstrual cycle effects on cortical excitation and inhibition later in the process of testing. Due to my own emerging results and other inconsistencies in the literature, I later discussed the available evidence of fluctuations in more detail (Krause & Cohen Kadosh, 2014). Such individual

fluctuations and variability affect both MRS and tES. Ideally, the phase of the menstrual cycle (early and late luteal and follicular phases) should be assessed for every female volunteer undergoing MRS or tES testing, albeit this might also raise some ethical concerns. One problem I have observed during the testing of my participants was that due to the testing of students at the University of Oxford, which involves high levels of stress, some women report to have abnormal menstrual cycles, e.g. have not had a menstruation period in several months. Similarly, most women participating in my studies use contraceptive pills, hormonal intrauterine devices (IUD coils), or hormone rings, which artificially modulate hormone levels and accordingly do not resemble normal levels of hormonal fluctuations. Therefore, my samples may also differ qualitatively from those investigated in the studies that found that brain stimulation and MRS effects fluctuate with the cycle (Epperson et al., 2002; Epperson et al., 2005; Finocchi & Ferrari, 2011; Harada, Kubo, Nose, Nishitani, & Matsuda, 2011; Hattemer et al., 2007; Smith, Adams, Schmidt, Rubinow, & Wassermann, 2002; Smith et al., 1999).

Lastly, optimal statistical power would require larger samples for most of the effects presented here. Due to practical difficulties in increasing the sample size, I have therefore reported effect sizes, used more robust non-parametric tests or covariates, and chose repeated measures designs. However, it is still unclear whether some of the null effects, including some correlational analyses, can be explained by a lack of adequate statistical power. Future studies should address this problem by using larger samples.

7.3 Future perspectives

Despite the current difficulties in the field to predict the effects of tES, it is a promising tool to enhance cognitive plasticity. Despite different ideas about mechanisms of action for tES (Bestmann, et al., 2015), MRS is currently one of the most useful tools to investigate the neurophysiological basis of changes induced by tES. Since simple motor studies have been relatively successful and consistent in relating neurochemical changes to tES, functional brain imaging, and performance, it can be helpful for the field of cognition to start systematic investigations using more simple cognitive domains or tasks. MRS

provides a unique and ideal method to combine neurobiological with higher-level cognitive research and induce and track changes using tES. Even though this approach is time-consuming and expensive, such integrative work is important to understand the interaction between pre-existing brain and behavioral features and the effects of non-invasive brain stimulation. In studies in which scans are acquired, the structural scan can also aid targeted electrode-fitting with neuro-navigation, in order to adapt the application of the current to the individual anatomy of each participant. This can be particularly useful for HD-tDCS or multifocal tES for the refinement of the current spread across the cortex, or to cover larger areas refined to a certain brain area (Datta, Baker, Bikson, & Fridriksson, 2011; Edwards et al., 2013). The localization of the electrical current, as well as the prediction of the functional response to the stimulation are the key concepts for successful stimulation, and the combination of computational modeling of current effects and deeper insight into the biological effects are important steps towards this success (De Berker, Bikson, & Bestmann, 2013). Such computational studies have provided valuable information about the effects of electrode size, shape, and position, the inter-electrode distance, the position of the reference electrode, different head sizes (including children) and influence of body fat in the head area, and individual variation in neuroanatomy (Bikson, Name, & Rahman, 2013; DaSilva, et al., 2011; Datta, et al., 2011; Datta et al., 2009; Datta, Truong, Minhas, Parra, & Bikson, 2012; Faria, Hallett, & Miranda, 2011; Im, et al., 2012; Kessler et al., 2013; Minhas, Bikson, Woods, Rosen, & Kessler, 2012; Moliadze, et al., 2010; Truong, Magerowski, Blackburn, Bikson, & Alonso-Alonso, 2013). Even though the tolerability of tES is generally good, even in children, it is important to further investigate how to avoid potential cognitive impairments (Andrade et al., 2013; Berryhill, Peterson, Jones, & Stephens, 2014; Brunoni et al., 2011; Mattai et al., 2011; Paneri et al., 2015). MRS is a promising tool to use neural markers for neurological impairments. Ideally, it would be possible to use glutamate and GABA as markers for poor cognitive abilities, which can distinguish the deficit based on concentrations in particular brain areas. At the group level, neuropsychiatric problems including autism, schizophrenia, depression, chronic pain, attention-deficit hyperactivity disorder (ADHD), Alzheimer's disease, Parkinson's disease and others can

already be distinguished using such MRS measures, some suggesting a role for E/I (e.g. Bai, et al., 2014; Brown, Singel, Hepburn, & Rojas, 2013; Emir, et al., 2012; Foerster, et al., 2012; Gaetz et al., 2014; Goto, et al., 2009; Kubas, et al., 2012; Perlov et al., 2007; Petrou et al., 2012; Rojas, et al., 2013; Rowland, et al., 2012; Rubenstein & Merzenich, 2003; Shaw et al., 2013; Yoon, et al., 2010). Such neuropsychiatric disorders are also associated with a certain degree of cognitive problems, such that it is likely that developmental learning difficulties may also be distinguishable from normal learners. First reports include promising findings to also link glutamate with poor reading abilities in children (Pugh, et al., 2014). Since tES has been suggested to be a valuable tool to improve poor reading abilities in children (Vicario & Nitsche, 2013a, 2013b), it would be interesting to also investigate the effects of tES and concurrent reading training on neurochemical changes. My current model of modulating existing neurochemical levels using tES paired with cognitive training can then ideally be targeted to remove the learning-related brakes on neuro-plasticity at both the neurobiological and the behavioral level.

7.4 Conclusion

Results of the experiments presented in this thesis revealed inconsistencies in certain behavioral effects of tRNS on arithmetic performance and at the same time failed to show changes in glutamate and GABA. TRNS is a promising tool to improve arithmetic learning and performance, but seems to also induce impairments in some cases. It is currently impossible to predict in which cases tRNS will be effective. Further work towards understanding the mechanism and developing optimized research strategies, as well as replicating existing studies across samples, is necessary. TES is therefore not yet ready for clinical use in cognitive (developmental) learning problems. However, side effects are reported to be minor and therefore the immediate tolerability of the stimulation appears positive. The effects of tES on cognitive functions appear largely positive, and have not been found to induce long-term impairments. However, conscientious research should involve long-term follow-ups. With the growing number of studies on cognitive improvement, this field is becoming wider but at the same time more comprehensive and integrated, and different angles are being combined in a more holistic understanding.

Across different experiments within this thesis, glutamate and GABA in certain regions of the mathematical brain network at baseline were significantly associated with basic mathematical abilities in different types of samples. This result fits in the current framework of linking such neurochemicals to higher-order cognitive abilities. Using non-invasive measures of neuro-physiological indicators for cognitive difficulties would be desirable for the diagnosis and subsequent consideration of potential intervention. Ideally, the increasing understanding of the links between glutamate and GABA and higher-level cognitive problems will lead to such possibilities.

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